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CORRECTION OF A REPORT

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A short time ago an exceptional report concerning the VIIth International Botanical Congress, which was held in Stockholm last summer, came to the notice of the present writers. This report, which appeared in the Tägliche Rundschau on Friday, September 15th, 1950, is written by Dr. I. E. GLUCHTCHENKO (И. Е. Глущенко), a member of the delegation of Soviet biologists attending the congress.[1] The report or parts of it have been re-published by several newspapers in Eastern Europe. Since this report is written in such a manner that the reader will necessarily obtain an entirely false impression of the congress, and especially where cytology and genetics are concerned, we consider it our duty as recorders of the two sections in question (CYT and GEN, respectively) to examine and correct at least some of the more flagrant statements of Dr. GLUCHTCHENKO. As the report also deals with an excursion of the Soviet biologists to various institutes in Southern Sweden in an equally unreliable manner, we shall also consider below this part of the report.

In the beginning of his report Dr. GLUCHTCHENKO writes among other things: »The members of the congress were offered more than 500 papers among which only few had any scientific interest». It should be noted that the congress papers were read in 15 different sections, which to a great extent were working simultaneously. Dr. GLUCHTCHENKO could therefore attend only a small part of these lectures. It is in keeping with the conscientiousness of Dr. GLUCHTCHENKO that he nevertheless feels quite at liberty to make the above sweeping judgment about all the papers read at the congress.

Dr. GLUCHTCHENKO continues: »The crisis of bourgeois theoretical botany was made especially evident by the fact that most genetical papers dealt with the production of those mis-shaped plants which are

[1] The report of Dr. GLUCHTCHENKO is reprinted *in extenso* as an appendix at the end of the present paper (pp. 301—305). An abbreviated reply to this report in the German language was sent by the present writers to the Tägliche Rundschau but has not yet appeared in this journal.

caused by mutations — sudden colchicine-induced changes of the hereditary material (Erbgrundlagen)». From the program of the congress it may readily be seen to what extent this statement of Dr. GLUCHTCHENKO is founded on facts. Among approximately 70 papers of the GEN section only four had any reference to colchicine-induced polyploids. That many papers both of the CYT and the GEN sections were devoted to the experimental induction of changes of the genetic constitution is, in our opinion, only natural. It is on such problems that the attention of the world's cytologists and geneticists is centred today. That these problems are of fundamental interest to the whole of biology is a fact which can hardly be disputed by anyone.

Dr. GLUCHTCHENKO may recall that during his visit to Svalöf after the congress the present writers had the pleasure of directing his attention to x-ray-induced mutations in barley and in other plants, as well as to colchicine-induced tetraploids of, among other things, red clover, alsike clover and rye. These new types are perfectly viable and have an undeniable practical significance. Within red clover, alsike clover and rye, several tetraploid types are at present propagated on a large scale. These types will be released for practical agriculture in the near future, the tetraploid rye already in this year. Thus it can be stated that after about twenty years of experimental work with the artificial production of mutants and polyploids the first practical results are now appearing. This is a good illustration of the fact that the new breeding methods, *viz.* mutation breeding and polyploidy breeding, are gradually taking their position besides the classical breeding methods.

The so-called classical breeding methods are, indeed, still of predominant importance. They imply selection and recombination within the existing genetic material. Thanks to the discoveries of MENDEL and the experimental evidence produced especially by NILSSON-EHLE, it is known that the practically important quantitative characters of plants are based on so-called polymeric genes, *i. e.* each property is governed by many cooperating genes. It is possible intentionally to combine certain valuable genes, and in that way produce new improved varieties of practical importance. According to Dr. GLUCHTCHENKO, the Soviet plant breeders perform »a directed education of the organisms, also those of hybrid origin»; in this way types with desired qualities are produced.

We note with interest that even in Russia plant breeders utilize organisms of hybrid origin. This may explain the success recorded from the selection work. As is well known there occurs in hybrid material a

great number of genic new-combinations which are the prerequisite condition for a genic adaptation to different environments. The »bourgeois» geneticists, far from denying the action of the environment in the evolution of new forms, have always pointed out the enormous importance of the environmental factors. They realize, however, that it is the interaction between recombination and environment that brings about the genetic adaptation and not the direct action of the environment.

The president of the congress, Dr. CARL SKOTTSBERG, maintained, according to Dr. GLUCHTCHENKO, »that the New World is leading in botany». Dr. GLUCHTCHENKO objects emphatically to this statement and pleads the following as counter-evidence: »What marvels then had the New World to exhibit at the congress? Professor BLAKESLEE, one of the pillars of MORGAN genetics, lectured about new hybrids of the Jimson weed. The theoretical significance of his paper is rather limited and it is certainly not worth while wasting one word on its practical importance».

Even at the risk of being called »lackeys of Wall Street», or something else of equal esprit, we wish to point out that the researches of BLAKESLEE have always been characterized by their immediate bearing on fundamental biological problems. His standing as a leading representative of genetics will hardly be jeopardized by the deplorable fact that he, as little as any other »bourgeois» geneticist, has never made any serious attempts to improve the cultural value of good old *Datura Stramonium*. The statement of Dr. GLUCHTCHENKO is interesting, however, because it shows him to have little appreciation of research work that does not immediately aim at practical applications.

The same thing is evident from the report of Dr. GLUCHTCHENKO concerning his visit to Svalöf. With the mordant irony, so characteristic of his literary style, he writes as follows: »At first we were shown the successes of Morganism in pure culture: miserable, low-growing rye and tall-growing rye poor in kernels. The first case is the result of a cross between related forms, not permissible from the standpoint of MICHURIN biology, the second case is explained by the action of colchicine».

It is absolutely correct that the former rye plants were on an average meagre and low; they belonged to a collection of inbred rye lines which is grown mainly for theoretical purposes. It has, however, escaped Dr. GLUCHTCHENKO that the most viable of these inbred lines are employed for the production of the highly fertile and vigorous rye-wheat hybrids. A number of such so-called *Triticale* types were demonstrated at Svalöf.

They represent a real synthesis of wheat and rye. In their nuclei there is present the sum total of the chromosomes of the parental species. Their somatic chromosome complement of 56 chromosomes consists of 42 wheat chromosomes and 14 rye chromosomes. They are constant types of good fertility, and their behaviour is in every respect that of a new species or that of a member of a new genus.

It cannot be denied that processes similar to those underlying the origin of *Triticale* have been of very great importance in the evolution of many higher plants. This is true not only concerning the wild flora but also to a great extent concerning the origin of many important cultivated plants, *e. g.* wheat and oats. The phenomenon of polyploidy, *i. e.* the occurrence of multiple series in the chromosome numbers of related types, is in principle well understood in terms of the chromosome theory. How is it possible that these facts can be compatible with the MICHURIN doctrine? The present writers would especially like to know whether the MICHURIN doctrine is able to give a better interpretation than the chromosome theory of the fact that primary species hybrids, when partially fertile, will give rise to a great variation in their progeny, while, on the other hand, after chromosome doubling, as in *Triticale*, the new products immediately acquire constancy and fertility. A beautiful case of this kind, now classical, was once closely analysed by G. D. KARPECHENKO, a compatriot of Dr. GLUCHTCHENKO, and the first scientific analysis of a *Triticale* strain was worked out by the Russian workers LEVITSKY and BENETZKAJA. It is deplorable that all these scientific facts are now turned down by Soviet geneticists, only because they are incompatible with MICHURIN's ideas.

It is also to be highly deplored that inbreeding, for exactly the same reason, is not accepted in Soviet Russia as an instrument in practical breeding. The Soviet state will suffer immense economic losses on account of this dogma. The great increase in yield attained by the so-called double-cross method, which has during recent years led to outstanding practical results in the U. S. A., above all within maize, will be lost to Soviet agriculture if the inbreeding of cross-fertilizers is not permitted. The exploitation of the increase in vigour arising after intentional intercrossing of selected inbred lines is a typical instance of how theoretical MENDEL-MORGAN-genetics and plant breeding in close cooperation may give results of the utmost importance to national economy and to human welfare. The utilization of the increase in vigour and yield after intercrossing inbred lines is, as is well known, not specifically valid only in maize: it may be generally applied to allogam-

ous plants. Similar breeding methods have, for instance, already for many years given prominent results in sugar beets here in Sweden.

Dr. GLUCHTCHENKO's report of the congress meeting at which members of the Soviet group read their papers is highly misleading in many respects. Although we feel it especially unpleasant to correct the report on these points, we nevertheless have to do so on account of several accusations levelled at other scientists by Dr. GLUCHTCHENKO in connection with this part of his report. The Soviet delegation arrived at the congress two days after its opening. Although they had not registered any papers in advance, the members of the Soviet group expressed their wish to read several papers. The organizing committee of the congress did its best to comply with this wish. Among other things, a special meeting was arranged within the GEN section. The only time available for this meeting was at 7 p. m. on Monday, 17th July. It was therefore necessary to include in this meeting all the four papers which the Soviet delegates wished to present in this section. Since, in addition, the papers were partly read in Russian and had to be translated into English, this meeting was very prolonged and did not end until after midnight. This is the background to Dr. GLUCHTCHENKO's report: »Not only the sectional meetings but also the plenary sessions ... finished as a rule at 6 or 8 p. m. Quite different was the situation when the Soviet scientists read their papers. Then the sessions were extended until after midnight». As the session in question started at 7 p. m., it would indeed have been surprising if it had finished at 6 or 8 p. m.

Dr. GLUCHTCHENKO further reports that the meetings of the congress were usually attended by only a few persons, while the auditorium during the Soviet lectures was filled unto the last place. It is correct that the special meeting in the GEN section was attended by a large audience. The main reason for this, however, was quite other than that assumed by Dr. GLUCHTCHENKO. We regret very much to have to disillusion Dr. GLUCHTCHENKO on this point. It is, however, a fact that the majority of the listeners, gathered at this meeting, had come not so much in the hope of learning new scientific facts or theories but in order to see in person and listen to people who deny even the most elementary facts of the science of genetics. Chemists, denying the existence of molecules or atoms, would undoubtedly attract an equally numerous audience, were they allowed to ventilate their opinions at an international chemical congress. The audience was also eager to hear how the members of the Soviet group were going to answer critical questions.

Unfortunately, the discussion after the Soviet papers was rather limited, partly because only written questions were considered. At first the Soviet speakers expressed a wish to postpone the answering of questions until the following day. Only after the protests of the audience against such an arrangement did the Soviet delegates agree to answer questions immediately. This readiness was applauded by the audience. The insinuations of Dr. GLUCHTCHENKO against the chairman, Dr. Ö. WINGE, in connection with this episode are thus perfectly groundless.

As to the questions, it must be said that they were not, or only partly, »sofort beantwortet». Some questions were not answered at all, as may of course occur in any discussion. With regard to other questions answers or attempts at answers were only obtained after a lapse of about two hours and after repeated restatement of the questions. With regard to this we have especially in mind a question asked by Prof. QUINTANILHA, and which is not mentioned in Dr. GLUCHTCHENKO's report. This question was formulated in about the following way: »In many lower plants it is possible to analyse separately the four cells of a single tetrad. In many such cases it has been possible to demonstrate that each two of the tetrad cells belong to a distinct category. If you deny the hereditary function of the chromosomes, how do you propose to interpret these facts?» After long consideration, and strongly pressed by his inquirer, Dr. GLUCHTCHENKO eventually conceded that »perhaps in this specific case the chromosome explanation would be applicable». We would be grateful to Dr. GLUCHTCHENKO were he to inform us what he thinks of the significance of the chromosome apparatus in other organisms. Why does this wonderful chromosome mechanism occur everywhere if it has no hereditary function?

Dr. C. D. DARLINGTON is mentioned repeatedly in Dr. GLUCHTCHENKO's report. Thus, for instance, it is stated that DARLINGTON suffered a moral defeat with his paper »the cell in the origin of life». Dr. GLUCHTCHENKO writes that DARLINGTON's paper »followed upon the genetical papers of the Soviet delegation. The congress regarded DARLINGTON as the main opponent of the Soviet delegates. Everybody expected with interest what important evidence he would mobilize against the biological doctrine of MICHURIN. DARLINGTON, however, continued to repeat the out-of-date theses of the cellular organization of the organisms . . .». It should be noted that DARLINGTON, on account of his prominent position as a cytologist as well as his eminent ability of presenting his research material in a manner understandable to those who are not especially trained in this branch of science, had been in-

vited by the organizing committee of the congress to read one of the very few so-called general papers. His paper (entitled »The study of the cell in the understanding of life») was delivered according to the program two days after the above-mentioned meeting with the Soviet delegates. It is consequently evident that DARLINGTON had no more reason to dwell upon the »biological doctrine of MICHURIN» than had all other congress members who were presenting papers after the Soviet meeting.

Dr. GLUCHTCHENKO complains that the »bourgeois» theoretical geneticists ignore and »kill by silence» MICHURIN's theories. We therefore wish to point out that in our personal opinion MICHURIN was without doubt a prominent and outstanding practical plant breeder responsible for a great number of highly valuable varieties of cultural plants, especially of fruit trees. His main breeding method was hybridization, particularly in forms which could be propagated vegetatively. We also know that MICHURIN associated his practical experiences with certain theoretical conceptions and speculations. As far as we can judge, these ideas have now only historical interest. In any case, they are without essential significance to the present genetical theories. MICHURIN has an American counterpart in LUTHER BURBANK, who, without close contact with genetical theory, was successful in producing many new varieties, especially of fruit trees.

Towards the end of Dr. GLUCHTCHENKO's report, under the heading »The MICHURIN biology makes school», there is a statement to the effect that after the visit to Svalöf a discussion between the Soviet delegates and the present writers showed »that the two scientists (MÜNTZING and LEVAN) »whole-heartedly agreed that the MICHURIN experiments cannot be explained on a Morganistic basis». In this connection certain experiments with vegetatively propagated rye are mentioned. The particulars of these experiments are unfortunately not quite clear from the report. We therefore must postpone our judgment about them until more detailed information is available. We both recall with great pleasure the very agreeable discussions we had with our Soviet colleagues. We recall that we pointed out on repeated occasions that experimental results which are apparently inconsistent with genetical theory are especially interesting and worthy of attention. »Treasure your exceptions», is rightly said by English and American scientists. Through the analysis of real or apparent exceptions many steps forward have actually been taken by science. A striking instance of this kind within our science is the *Oenothera* problem, which for a long time remained

a puzzling exception. Over a period of many years, and through the combined efforts of numerous workers, the riddle of *Oenothera* was, however, solved. Both cytological and genetical theory gained greatly by the solution of this problem.

We wish to declare that the fact that we, as well as other »WEISMANN—MENDEL—MORGAN geneticists», admit that the present genetical theories are not capable of immediately classifying and »explaining» all empirical data, cannot be used as evidence that we are »Michurinists», as actually hinted in Dr. GLUCHTCHENKO's report. It is astonishing that Dr. GLUCHTCHENKO obtained this impression in spite of the fact that during our discussions at Lund we repeatedly and urgently tried to make clear that the ideas which the Soviet delegates had formed about us »Western» geneticists — our way of thinking, our opinions in science, our material of knowledge, etc. — were often extremely erroneous and coloured by prejudice. Western geneticists do not believe that the genes and the chromosomes are alone responsible for everything in heredity. We certainly do not deny that environment plays a great rôle in biological variation. Still, without being Michurinists, we know that many external agents are capable of inducing changes in the genes and the chromosomes, thus directly affecting heredity. It is a significant fact that the line of research at present most actual within Western cytogenetics is the study of such external agents as may induce hereditary changes.

In addition, all Western geneticists agree that components of the cell, other than the chromosomes, may be carriers of heredity. Especially in recent years attention has often been directed towards extra-nuclear hereditary factors, plastids, mitochondria, plasmagenes, etc. We hope that Dr. GLUCHTCHENKO has taken advantage of the list of literature on plasmatic inheritance sent to him a few months ago by one of us.

We have tried above to make a pertinent analysis of some points in the report of Dr. GLUCHTCHENKO. Before finishing, the present writers wish to point out that they hold in very high esteem the acumen and originality of Russian thought. We are convinced that Russia's biologists, now as before, will continuously obtain results from their researches which will prove indispensable to the development of the biological science of the entire world. On the other hand, Soviet biology can only lose by isolating itself from the so-called Western biology. It is highly

deplorable that a scientist of Dr. GLUCHTCHENKO's standing allows himself — as in the report about the Stockholm congress — to show only contempt towards the results of the many thousands of sincere and devoted biologists of the »Western» world.

All biologists are confronted with the extremely complicated and intriguing problems of living matter. We must all admit that our intellectual powers are much too limited to grasp even a small part of this complexity. Still, there is no doubt that we will have more success in our efforts, if only all good forces could cooperate instead of spending time, interest, and energy on sterile polemics and propaganda.

In spite of the disappointment we have felt on finding that our reception of the Soviet delegates in Stockholm and in South Sweden has resulted in such an obviously malevolent document as the report of Dr. GLUCHTCHENKO, we are still prepared to welcome Soviet cytologists and geneticists as guests at our genetical institutes: we still wish to discuss freely questions of our work with them; and we still gladly put at their disposal living experimental material, as well as undertake an exchange of our scientific publications.

We would appreciate an answer from Dr. GLUCHTCHENKO with regard to our remarks. The Editor of Hereditas has consented to the columns of this Journal becoming the medium for such an answer, provided the latter is scientific and does not require too much space. We consider a pertinent discussion of the problems involved to be of the utmost importance.

APPENDIX.

DR. GLUCHTCHENKO'S REPORT.

Auf dem VII. Internationalen Botanikerkongress traten sehr deutlich zwei Richtungen in der Wissenschaft zutage: die reaktionäre der Anhänger Weismanns und Morgans und die fortschrittliche der Mitschurin-Anhänger. Den Kongressteilnehmern wurden mehr als 500 Referate geboten, von denen aber nu wenige wissenschaftlich von Interesse waren. Besonders deutlich offenbarte sich die Krise der bürgerlichen theoretischen Botanik darin, dass die meisten genetischen Referate sich mit den Methoden zur Erzeugung von missgestalteten Pflanzen beschäftigten, wie sie durch Mutationen — plötzliche, durch Kolchizin herbeigeführte Veränderungen der Erbgrundlagen — hervorgerufen werden. Von diesem Gift machen die Morganisten seit einigen Jahren viel Wesens.

Nach Morgan lässt sich die Erblichkeit eines Organismus durch aussergewöhnliche Einwirkungen verändern, z. B. durch stark wirkende Gifte oder sehr starke Röntgenbestrahlung. Diese morganistischen Mutationen ergeben zufällige Formen,

sie sind eine Art Lotteriespiel. Selbst wenn es gelingt, dabei Pflanzen auszuwählen, die für den Experimentator von Interesse sind, so ist doch ihre Fruchtbarkeit gewöhnlich sehr gering.

Nach Mitschurin ändert sich die Erblichkeit durch die Lebensbedingungen. Durch gelenkte Erziehung der Organismen, auch derjenigen von hybrider Abstammung, erzielen wir Arten mit gewünschten Eigenschaften. Mit Hilfe dieser Methode können die sowjetischen Pflanzenzüchter die Geographie der Flora ihres Landes umgestalten.

Die »Errungenschaften» der Morganisten. — Obwohl dieser Streit der beiden Richtungen in der Wissenschaft längst durch das Leben entschieden ist, versuchen die bürgerlichen Theoretiker, die Mitschurinsche Lehre zu ignorieren. Der Präsident des Kongresses, Professor Scottsberg, wollte die Delegierten durchaus davon überzeugen, dass die Botanik der Vereinigten Staaten eine »Feste» sei, »die keinem das Feld räumt». »Es ist zwecklos, bestreiten zu wollen», sagte er, »dass die Neue Welt in der Botanik die Führung hat.»

Was für Merkwürdigkeiten hatte die Neue Welt auf dem Kongress zu bieten? Professor Blacksly, eine Säule der morganistischen Genetik, referierte über neue Hybriden des Stechapfels. Die theoretische Bedeutung seiner Arbeit ist sehr begrenzt, und über die praktische lohnt es sich nicht, ein Wort zu verlieren. Die Delegierten brachten ihre Einschätzung seiner Entdeckungen dadurch zum Ausdruck, dass sie während seines Referats dem Konferenzsaal fernblieben.

Eine moralische Niederlage erlitt auch Professor Darlington, das Haupt der englischen Morganisten. Sein Vortrag über das Thema »Die Zelle bei der Entstehung des Lebens» folgte auf die genetischen Referate der Sowjetdelegation. Der Kongress sah in Darlington den Hauptopponenten der sowjetischen Delegierten. Jeder wartete mit Interesse darauf, welche gewichtigen Argumente er gegen die biologische Lehre Mitschurins vorbringen werde. Darlington aber wiederholte nur die veralteten Thesen vom zellularen Aufbau der Organismen. Mit keinem Wort erwähnte er die neuesten Entdeckungen der sowjetischen Zytologen und Mikrobiologen O. Lepeschinskaja, G. Boschjan und anderer, durch die die idealistische Vorstellung Virchows von der Zelle als dem einzigen Ausgangspunkt alles Lebendigen widerlegt wird.

Nicht eine einzige Frage wurde an den Referenten gerichtet. Dafür sprach man in den Wandelgängen darüber, die langweilig, uninteressant und rückständig seine Ausführungen gewesen seien.

Einige Botaniker hielten derartig lebensfremde und unwissenschaftliche Referate, dass man sich wundern musste, wie sie auf die Tagesordnung des Kongresses geraten konnten. Sie demonstrierten einzig den Wunsch gewisser Verfasser, sich den Vertretern der USA gefällig zu erweisen. So referierte z. B. ein Professor Walter aus Westdeutschland über seine vor 18 Jahren durchgeführten Arbeiten zur Erforschung des Wasserhaushalts der Pflanzen in der Arizonawüste. Derselbe Walter war während der Besetzung der Ukraine durch die Nazis bemüht, dort die faschistische »Wissenschaft» heimisch zu machen.

Die Sowjetwissenschaftler sprechen ... — Die Referate der Sowjetwissenschaftler standen im Mittelpunkt der allgemeinen Aufmerksamkeit und bewiesen die Ueberlegenheit der Wissenschaft der Sowjetunion über die der kapitalistischen Länder. Der sowjetische Delegationsführer, Akademiemitglied W. Sukatschow, gab einen Ueberblick über den gewaltigen Umfang der in den letzten 20 Jahren in der Sowjetunion geleisteten botanischen Arbeit. Alle diese Leistungen seien der entscheidenden

Aufgabe untergeordnet, die Natur zum Wohle der Werktätigen umzugestalten. Er berichtete über die Arbeiten zur Erforschung der Pflanzendecke und zur Kartographie der Vegetation in der Sowjetunion. In dieser Hinsicht ist die Sowjetunion den anderen Ländern weit voraus. Die gleiche enge Verbindung von Theorie und Praxis und die Mitwirkung der Wissenschaft an der Erfüllung der grossen Volkswirtschaftsaufgaben spiegelte sich auch in den übrigen Referaten und Ausführungen der Sowjetwissenschaftler wider.

Nicht nur die Sitzungen der Sektionen, sondern auch die Plenarsitzungen waren im allgemeinen von nur wenigen Delegierten besucht und endeten in der Regel um 6 oder 8 Uhr abends. Anders sah es aus, wenn die Sowjetwissenschaftler sprachen. Dann zogen sich die Sitzungen bis nach Mitternacht hin, und trotzdem hatten wir nicht über Mangel an Zuhörern zu klagen. Selbst die sozialdemokratische »Morgon Tidningen», der man wirklich keine Sympathie für die Sowjetwissenschaft nachsagen kann, berichtete am 18. Juli, die Räume seien, wenn die sowjetischen Delegierten gesprochen hätten, bis zum letzten Platz gefüllt gewesen, und gelegentlich hätten Zuhörer auf dem Fussboden sitzen müssen.

Unsere vier Referate über Probleme der Mitschurinschen Genetik — von den Professoren W. Stoletow, N. Turbin, K. Suchow und dem Verfasser dieser Zeilen — wurden übrigens in der Sektion für Genetik gehalten. Die Zahl der Zuhörer aber ging in die Hunderte, und es waren nicht nur Genetiker, sondern auch Vertreter anderer Zweige der Botanik. Zahlreiche Fragen wurden an uns gerichtet.

Verleumdungen brechen zusammen. — Prof. Darlington suchte durch Zwischenfragen bei den Zuhörern den Eindruck zu erwecken, als ob die sowjetischen Selektionäre sich mit fremden Federn schmückten. Diese Unverschämtheit erstaunte uns nicht im geringsten. Schon im Februar 1947 hatte Darlington in der Zeitschrift »Discovery» versichert, das ganze Verdienst Mitschurins bestehe in der Akklimatisierung kanadischer und nordamerikanischer Obstbaumarten. Das wagte Darlington zu behaupten, während die Amerikaner und Kanadier noch lebten, die sich Mitschurins Züchtungen aus Koslow besorgt und sie als die besten der Welt bezeichnet hatten. Es macht Darlington auch nichts aus, dass ein kanadischer Farmerkongress vor 52 Jahren feststellte, in strengen Wintern seien alle amerikanischen und europäischen Kirscharten erfroren, und nur Mitschurins »Plodorodnaja» haben standgehalten.

Dies und vieles andere legten wir bei der Beantwortung der an uns gerichteten Fragen dar. Bemerkenswerterweise kam es vorher zu einem interessanten Zwischenfall. Um Darlington zu unterstützen, erklärte der den Vorsitz führende Däne Winge, die Russen wollten die an sie gerichteten Fragen nicht sogleich beantworten, sondern würden das vielleicht ein andermal tun. Das war ein offensichtlich antisowjetisches Manöver. Im Saal verbreitete sich das Gerücht, die sowjetischen Botaniker müssten ihre Antworten mit Moskau abstimmen. Wir haben selbstverständlich alle Fragen sofort beantwortet.

»Diese Sitzung», schrieb die Zeitung »Ny Dag», »wurde zum glanzvollen Höhepunkt des Kongresses. Die russischen Professoren erläuterten die grossartigen Erfolge der Sowjetwissenschaft und ihre epochemachenden Entdeckungen.»

Viele Zuhörer hörten aus unseren Referaten zum erstenmal etwas von Mitschurin und Lyssenko, von ihrer Lehre, von den grandiosen Arbeiten zur Umgestaltung der Natur in der Sowjetunion.

Die Lakaien der Wallstreet und der City suchen mit allen Mitteln zu verhindern, dass die Wahrheit über die Mitschurinsche Wissenschaft unter den Gelehrten

der kapitalistischen Länder bekannt wird. Wenn es aber unmöglich wird, die Lehre Mitschurins und die Forschungen Lyssenkos und anderer Sowjetwissenschaftler totzuschweigen, dann sucht man sie zu verzerren. Die Morgan-Anhänger haben, um Lyssenkos Anschauungen zu banalisieren, vielen Wissenschaftlern eingeredet, wir leugneten die Existenz der Chromosomen, jener unter dem Mikroskop sichtbaren Zellenteilchen, deren Anzahl für jede Tier- und Pflanzengattung verschieden ist. Aber auch diese Lüge wurde entlarvt.

Während des Kongresses konnten wir uns davon überzeugen, dass viele ausländische Wissenschaftler sich lebhaft für die Sowjetwissenschaft interessieren. Es verdient erwähnt zu werden, dass mehrere Wissenchaftler Schreiben an uns richteten, in denen sie sich für die interessanten Referate bedankten.

Als handgreifliches Beweisstück für die gewaltige Entwicklung des theoretischen Denkens in der Sowjetunion überreichten wir dem Kongress das 15bändige Werk »Die Flora der Sowjetunion», das in der Welt nicht seinesgleichen hat. Es wurde mit Dankbarkeit entgegengenommen.

Die sowjetischen Botaniker haben in Schweden nicht nur auf dem Kongress selbst gesprochen. Auch auf Mitgliederversammlungen der Schwedisch-Sowjetischen Gesellschaft in Stockholm, Göteborg und Lulea haben wir Vorträge gehalten.

Mit Vergnügen erinnern wir uns mehrerer freundschaftlicher Begegnungen mit schwedischen Wissenschaftlern und breiteren Kreisen der Oeffentlichkeit, die der Schwedisch-Sowjetischen Gesellschaft nahestehen.

Die Mitschurinsche Biologie macht Schule. — Die Kongressteilnehmer besuchten das Institut für Genetik an der Universität Lund und die berühmten Versuchsstationen in Svalöv und Landskrona. Zunächst zeigte man uns Errungenschaften des Morganismus in Reinkultur: mageren, niedrigen und hochwüchsigen körnerarmen Roggen. Der erste Fall ist das Ergebnis einer vom Standpunkt der Mitschurinschen Biologie aus unzulässigen längeren Kreuzung nahe verwandter Arten, der zweite erklärt sich durch die Wirkung des Kolchizins. Auch mit Röntgenstrahlen behandelte Gerste wurde uns vorgeführt. Sie sah keineswegs besser aus als die Ausgangsformen.

Dann führte man uns, um den fatalen Eindruck dieser Kollektion von biologischen Missformen abzuschwächen, auf prächtige Weizenfelder. Die hier wachsenden Sorten sind durch die übliche Kreuzung hervorragender Sorten und darauf folgende Auswahl gewonnen worden.

Ein bemerkenswerter Kontrast! Die Formalgenetiker erhalten durch die Anwendung ihrer Theorien in der Regel nichts als Missformen. Wenn aber irgendeine lebenswichtige Aufgabe zu lösen ist, denken sie nicht mehr an den Morganismus.

Nach der Besichtigung der Versuchsstationen entspann sich zwischen uns und den schwedischen Gelehrten Arno Munzing und Albert Lewan eine Diskussion über die Grundfragen der Mitschurinschen Biologie. Wir bewiesen ihnen die Lebensfähigkeit der Mitschurinschen Biologie an Hand von zahlreichen Beispielen für die Unhaltbarkeit der Chromosomentheorie in der Erblichkeitslehre und durch die Erfolge der praktischen Anwendung von Mitschurins Lehre. Beide Gelehrte gaben offenherzig zu, dass sich die Mitschurinschen Experimente morganistisch nicht erklären lassen. Insbesondere nützte ihnen die formale Genetik auch für die theoretische Analyse eines unserer Experimente nichts: Wir säen im Frühjahr ein Winterroggenkorn aus und erhalten eine üppige Staude. Sodann zerschneiden wir die Wurzeln samt den Halmen in viele Teile und verpflanzen sie in einzelne Gefässe. So erhalten wir bis zu 200 Pflanzen aus einem einzigen Korn! Jede Pflanzengruppe düngen wir nun verschieden.

Die verschiedenen Existenzbedingungen verwandeln die 200 Nachkommen eines und desselben Organismus gewissermassen in verschiedene Organismen. Ein Beweis dafür ist die normale Samenentwicklung dieser Pflanzen in der Blütezeit. Sie alle ergeben eine gesunde Nachkommenschaft. Die Schweden interessierten sich sehr für dieses Experiment, versprachen, es bei sich zu wiederholen, und mussten abermals zugeben, dass es sich morganistisch nicht erklären lässt.

Es ist kein Zufall, dass sich ein Kollege und Landsmann unserer Gesprächspartner, Prof. Herbert Nilson, nach langjährigen Versuchen, neue Roggensorten mit den Methoden der morganistischen Genetik zu züchten, von diesen Methoden abgewandt hat. »Meine umfassenden Versuche, diesen Weg der Selektion zu gehen, sind vollständig gescheitert», schrieb Nilson.

Unter den Formalgenetikern des Auslandes ist eine Spaltung festzustellen. Man kann die reaktionären Wissenschaftler, die dem Imperialismus mit Leib und Seele dienen und die Sowjetwissenschaft zu diffamieren suchen, nicht mit der Masse der Botaniker des Westens gleichsetzen.

Die entscheidende Bedeutung des Kongresses besteht darin, dass er den Nebel, in den die Morgan-Anhänger die Mitschurinsche Lehre hüllen, grossenteils zerstreut hat. Obwohl die kapitalistischen Staaten bestrebt waren, die von ihrem Standpunkt aus zuverlässigsten Leute auf den Kongress zu schicken, bekundeten viele von diesen ehrliches Interesse für das, was wir zu sagen hatten.

Viele Biologen des Westens beginnen einzusehen, dass die Lehren Weismanns und Morgans widerlegt sind. Sie erkennen die Kraft und Wirksamkeit der Mitschurinschen Biologie. So erklärt sich das ungewöhnliche Interesse dieser Gelehrten für die Referate der sowjetischen Delegierten auf dem Kongress.

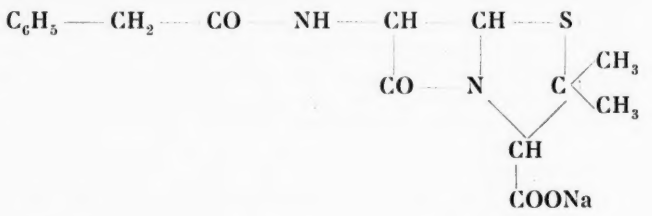
Der VII. Internationale Botanikerkongress wird in die Geschichte der Biologie als der Kongress eingehen, auf dem die Armseligkeit des Morganismus und die Lebenskraft der Sowjetwissenschaft demonstriert wurde, die dem Sowjetstaat hilft, unter der Führung der Kommunistischen Partei die Höhen des Kommunismus zu erreichen.

PENICILLIN IN THE ALLIUM TEST

BY *ALBERT LEVAN* AND *JOE HIN TJIO*
CYTO-GENETIC LABORATORY, SVALÖF, SWEDEN

IN a lecture at the Mendelian Society, Lund, on May 13th, 1949, Professor WESTERGAARD mentioned having observed a weak mutagenic activity of penicillin in *Neurospora*. In view of the great practical importance of this substance it seemed to be of interest to study its influence on root chromosomes of *Allium Cepa* with the object of ascertaining any chromosome-breaking activity it may have. Experiments were planned for complete *Allium* tests and — thanks to a generous supply of penicillin presented by the firm AB Kabi, Stockholm (formerly known as Kärnbolaget) — the present study could be started.

The penicillin employed is manufactured by AB Kabi and consists of practically pure sodium benzyl-penicillin of the formula:



The molecular weight is 356,37, one million international units (MIU) (0,6 g) corresponding to 0,00168 mol. Our experiments include the concentration range 0,001—20 MIU per 100 cc, *i. e.* 0,000017—0,336 mol/l. Throughout the following paper all concentrations are given only as the number of MIU per 100 cc. The pH of the solutions was often controlled and was nearly 7.

The experiments were conducted in the ordinary way (LEVAN, 1949). Treatments were made in 70 cc test tubes, except the strongest, for which 10 cc tubes were employed. During the autumn of 1949 three experimental series, and during the autumn of 1950 a further two series were made. Series with continuous treatment as well as series with short treatment followed by recovery in water were arranged. Root tips were fixed at various intervals after the start of the treatment. The experi-

ments were ordinarily observed for three weeks. The following experiments were performed:

Experiment 1 (started $^{11}/_{8}$, 1949). Concentrations: 0,004—2, totalling 10 concentrations. Four days' treatment, whereupon the bulbs were transferred to water.

Experiment 2 (started $^{18}/_{8}$, 1949). Concentrations: 2,5—10, totalling 3 concentrations. Four hours' treatment, then over to water.

Experiment 3 a and b (started $^{16}/_{9}$, 1949). Concentrations: 0,02—20, totalling 10 concentrations. Treatment (*a*) 2 hours, (*b*) 4 hours, thereupon to water.

Experiment 4 a and b (started $^{2}/_{8}$, 1950). Concentrations: 0,1—20, totalling 8 concentrations. Treatment (*a*) continuous, (*b*) 4 hours.

Experiment 5 a and b (started $^{18}/_{10}$, 1950). Concentrations: 0,001—1, totalling 10 concentrations. Treatment (*a*) continuous, (*b*) 5 days.

Although the results were somewhat varying between different series, they were concordant enough to be treated together in the following report. The results from all experiments are collected under three headings: (I) Lethality and toxicity — recording the effects of strong lethal or toxic concentrations, (II) Narcotic cell reactions, and (III) Radiomimetic effects.

Our thanks are due to Professor M. WESTERGAARD for putting us on the trail of this investigation and to Mr. B. ISAKSSON, Manager of the AB Kabi, for providing us with the substance used.

I. LETHALITY AND TOXICITY.

1. MORPHOLOGICALLY.

Only in the strongest concentrations employed (0,5—20) were toxic effects of the penicillin met with, such as disappearance of the turgor of the root tips (lethality) and inhibition or retardation of longitudinal growth (toxicity). Cytologically, the lethality and severe toxicity were

Concentration	Duration of treatment
20	2 hours 30 min.
10	1 day (24 hours)
5	2 days
2	2 »
1	4 »
0,5	roots turgescent during the whole experiment

associated with drastic nuclear changes, nuclear fragmentations, pychnosis, and chromosome stickiness.

On continuous treatment the turgor disappeared under the following conditions (experiment 4 *a*) [see preceding page, below].

During the various recovery treatments the following observations were made:

2 hours' treatment: Concentration 20: soft after 48 hours; 10 kept the turgor all the time.

4 hours' treatment: Concentration 20, soft after 48 hours; 10, after 3 days; 5, turgescent all the time (experiment 3 *b*). In experiment 4 *a*, concentration 20 already gave soft roots after 2,5 hours' treatment, as reported above. In experiment 4 *b* the roots began softening in concentration 5 after 4 days, while the roots in concentration 2 kept turgescent the whole time.

4 days' treatment: Concentration 2, soft after 4 days' recovery; concentration 1, turgor all the time.

Summing up, it may be stated that the lowest lethal dosages of penicillin in *Allium Cepa* roots are the following. Concentration 1 in continuous treatment, 2 in four days' treatment, 5 and 10 in 4 hours', and 20 in 2 hours' treatment. There is a very strong increase in the toxicity between concentrations 1 and 5.

The influence of penicillin on the longitudinal growth of the roots was observed in all series. It was found that growth retardation occurred in the strongest concentrations down to 0,5 under continuous treatment. Growth was completely suppressed in and above concentration 2. In experiment 5, which was especially designed to elucidate the growth conditions in different concentrations, daily measurements of the root length were made. In the *a* series (continuous treatment, solutions renewed on the 4th day) the two strongest concentrations 1 and 0,5 showed growth retardation all the time. The growth curves of all the other concentrations run rather close together. The curves of the four controls were situated in the upper half of the swarm of curves. Thus, there was no sign of growth stimulation by the weaker dosages of penicillin. On the 7th day, for instance, the root lengths were as follows:

	Penicillin										Controls			
Concentrations	1	0,5	0,2	0,1	0,05	0,02	0,01	0,005	0,002	0,001	0	0	0	0
Length in mm	41	43	77	82	72	77	86	81	72	87	92	94	95	85

In the *b* series (treatment for four days, then transference to water) the results were similar though less regular. Concentration 1 had less growth than the others, which were more scattered than in the *a* series.

On the 7th day the curves were counted from below in the following order: 1, 0,1, 0,02, 0,5, 0,2, the control, 0,01 and 0,05. Concentrations 0,005 and 0,001 were causing putrefaction on the 6th day and 0,002 already on the 5th day. It should be noted that the only bulbs attacked by rot in this experiment were those treated by the three lowest concentrations. The attack occurred soon after the transfer from penicillin to water.

2. CYTOLOGICALLY.

The strongest concentrations of penicillin induced characteristic cytological changes. A description will be given here in some detail of a reaction which, although in all probability not specific to penicillin, manifested itself in our experiments with unusual clearness and therefore deserves closer attention. This reaction was regularly present in concentrations 10 and 20. In its extreme effects it involved a disintegration of the entire nucleus into a great many chromatin droplets. The different stages of this reaction could be clearly followed in our treatments.

Fig. 1 presents two pictures of the general appearance of this reaction when it is rather weakly developed. Almost all nuclei in the meristem are provided with one, two or more protuberances of very characteristic type. These are mushroom-shaped and consist of a rounded externally concave disk, often strongly chromatic, and a rather narrow stalk varying in length. The stalk is more weakly stained and often exhibits longitudinal striation. In slides stained with crystal violet (Figs. 1 and 2) the disk of the protuberances almost invariably shows a deeper colour than the rest of the nucleus. It seems as if a large part of the chromatin is gathered to these parts. In order to ascertain that really chromatin and not nucleolar substance is responsible for this staining Feulgen tests were made. Fig. 3 shows the result: the protuberances are still strongly stained and are thus chromatic. In certain cases the nucleoli may be directly observed in nuclei with protuberances.

In Fig. 2 the variation in appearance of these protuberances may be seen: they may be of different size in the same nucleus (Fig. 2 *f*) and they may be branched (Fig. 2 *d*).

Particles in the plasm scattered out as a funnel-shaped cloud outside the disk indicate that they sometimes contain a crater through which nuclear contents are extruded into the plasm (Fig. 2 *a*). The details of Fig. 3 are suggestive: *a*, *b* and *c* form a series in which a protuberance is formed whose stalk is stretched out and whose disk finally loosens. At the same time as the disk is liberated its shape changes, it is rounded

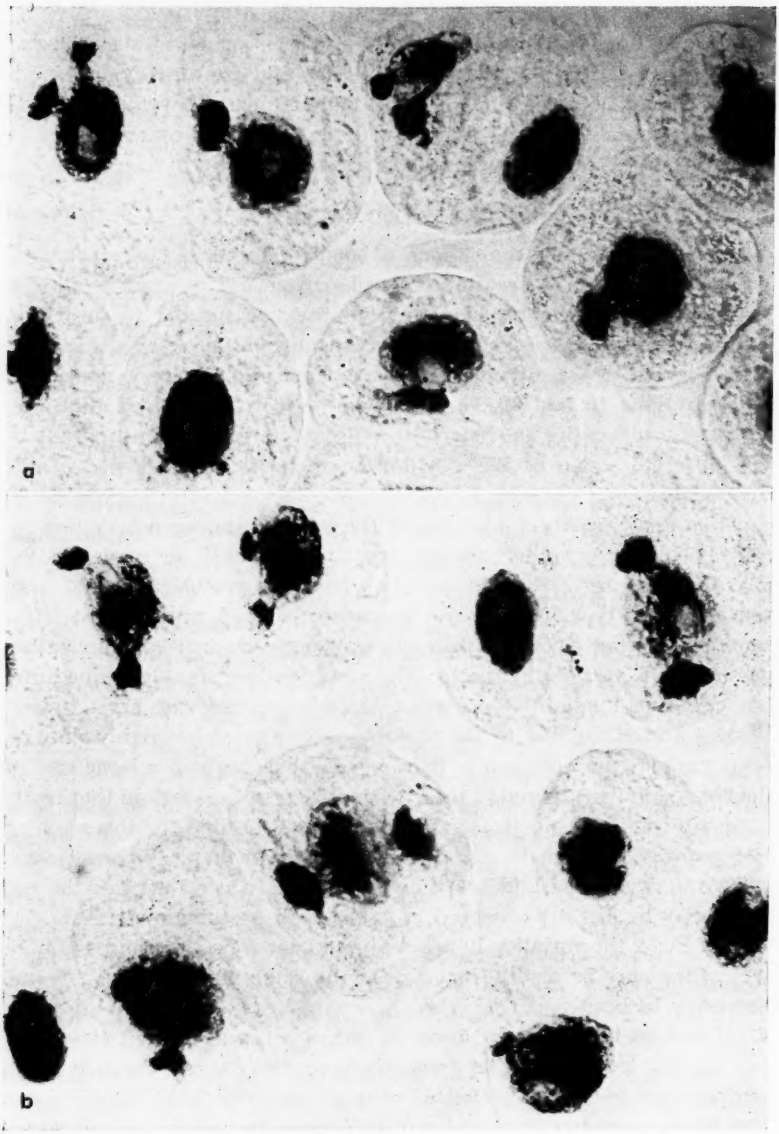


Fig. 1. *a, b*: The general appearance of the nuclear reaction to strong penicillin dosages.

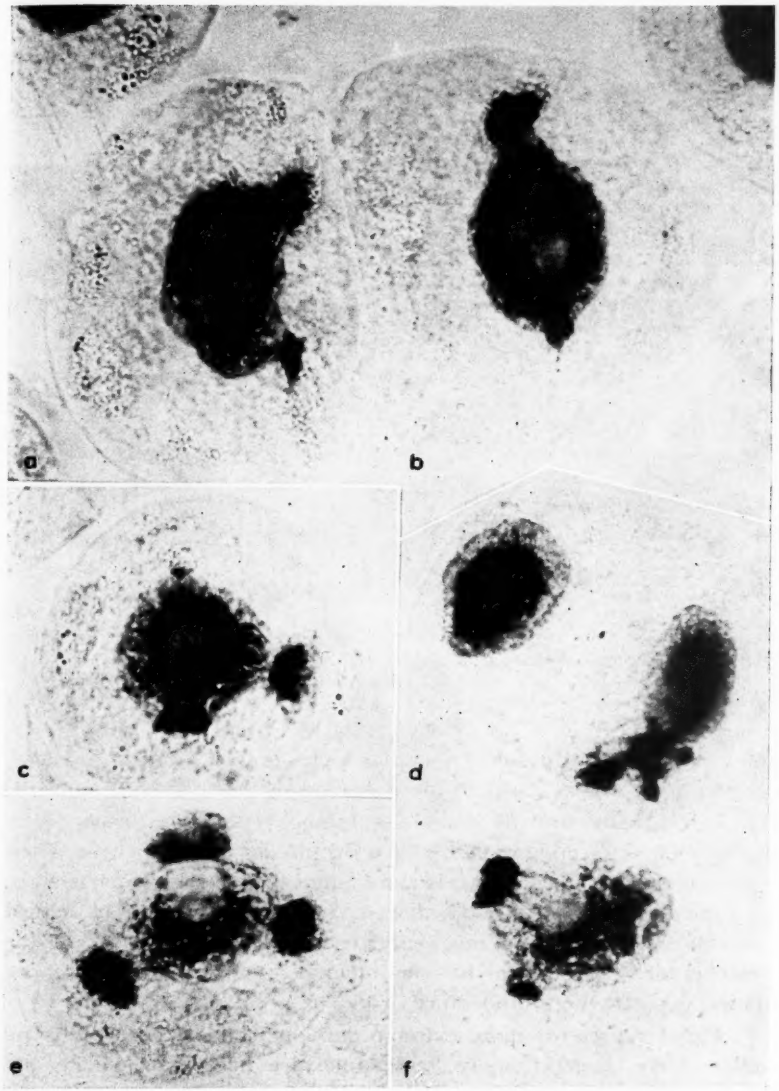


Fig. 2. The appearance of the penicillin-induced protuberances of the nuclei. Crystal violet.

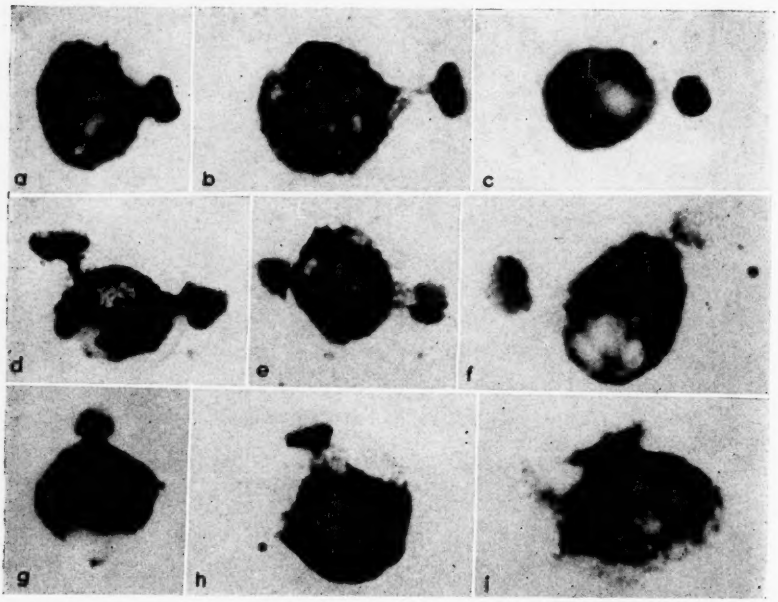


Fig. 3. The appearance of the protuberances after Feulgen staining. *a—c*: three stages in the complete loosening of a nuclear bud; *d—f*: the same with two buds; *g—i*: bursting of the protuberances.

off into a spherical droplet. A similar series is formed by Fig. 3 *d—f*, which show various stages in the liberation of two nuclear fragments.

Evidently the strongly stainable substance is present in the periphery of the nucleus, forming a shell around the interior of the nucleus. When a protuberance is formed, it gets chromatin in its peripheric parts while its contents are more weakly stained. Sometimes the weakly stained contents can be seen to flow out laterally around the stalk of the protuberance (Fig. 3 *h*) or to some other place in the nucleus, for instance, opposite the protuberance (Fig. 3 *g*).

Fig. 4 represents more extreme developmental stages of this reaction. Here, a great many Feulgen-positive nuclear fragments are present; in extreme cases the whole nucleus may be atomized into many fragments. That it is only a question of the final stage of the same process as described above, is shown by the many transitional stages seen. For instance, Fig. 4 *c* shows one big crater-shaped protuberance,

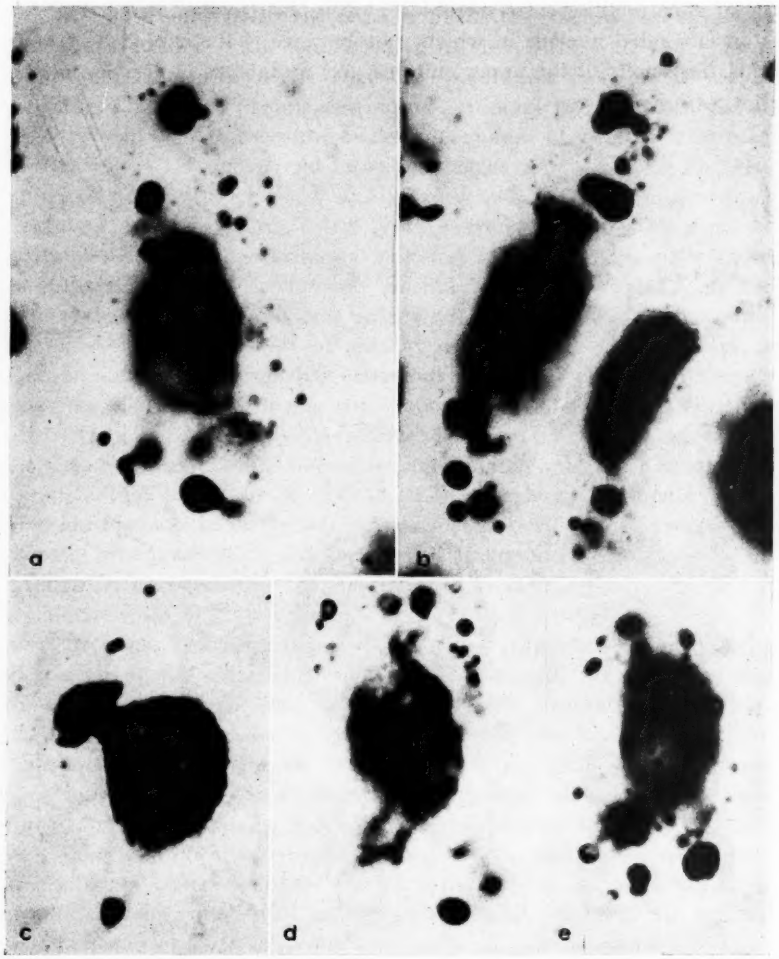


Fig. 4. Extreme fragmentation of nuclei after penicillin treatment. In *b* there is seen a crater through which nuclear material is thrown out. Feulgen.

which has thrown out around its orifice a couple of larger droplets and many smaller ones, and one small protuberance already loosened from its stalk and just beginning to get rounded off. Its origin may be directly observed, since its path through the plasm is marked by an, at least on

the original picture, clearly visible Feulgen-positive shadowing. Fig. 4 *b* is an elongated nucleus in which protuberances have exploded at both ends. Especially at the upper end one gets an intense impression of the

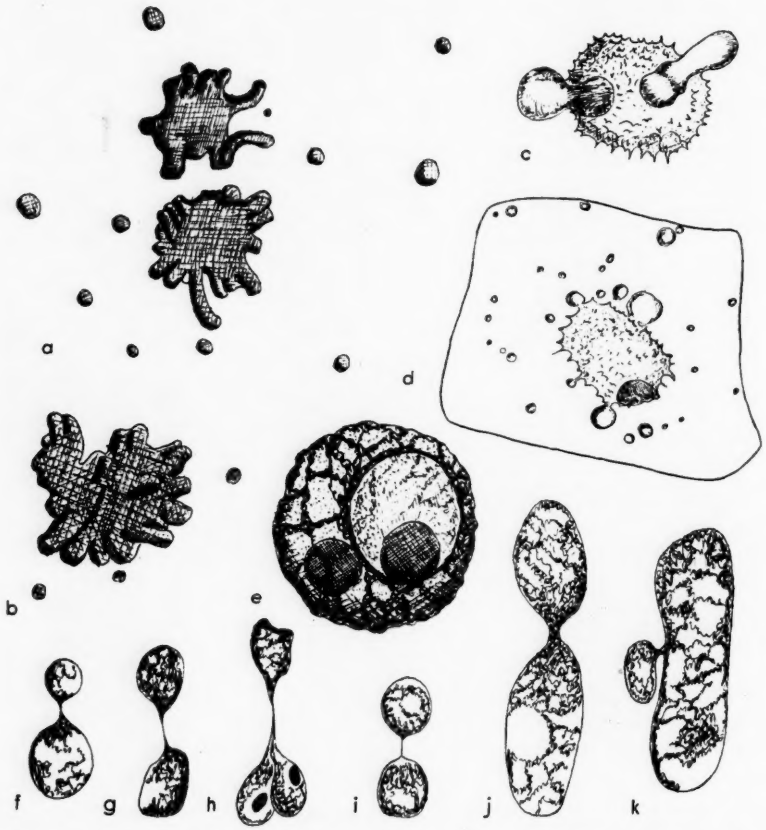


Fig. 5. Severe penicillin damages. *a*: anaphase with many chromatic bodies; *b*: metaphase; *c*: resting nucleus with protuberances; *d*: ditto with numerous scattered chromatic bodies; *e*: hollow resting nucleus; *f—k*: »amitotic» nuclei. — *a—e*, × 1500; *f—k*, × 700.

power in action when the protuberance has burst and the larger and smaller droplets have been slung out into the plasm. They form an ovoid cloud in front of the funnel. Other such funnels with their extrusions are shown in Fig. 4 *a*, *d* and *e*.

In Fig. 5 some lethal reactions of nuclei are drawn. Fig. 5 *c* and *d* are nuclei treated with concentration 20 for three and four hours, respectively (experiment 4); *c* has two protuberances but as yet no droplets, *d* has many protuberances and chromatic droplets scattered all over the cell. In both cases the nuclei are echinate all over their surface. In some cases the spines end in small vesicles which may be liberated into free droplets. Fig. 5 *a*, *b* and *e* are drawn from the next weaker treatment, 10 for 4 hours. *e* is a typical resting nucleus. There are no protuberances but the chromatic material forms a coarse network at the internal layer of the nucleus, its interior is hollow and there are two nucleoli present. This is a somewhat weaker lethal change. Surprisingly enough, this type of nucleus seems sometimes to be able to recover and return to normal behaviour. Fig. 5 *a* and *b* show the occurrence of fragments during contraction stages of mitosis. They may often originate through the liberation of liquefied matrix substance from the chromosomes. Similar phenomena were observed at salt treatments, for instance lithium nitrate (LEVAN, 1949). Anyhow, fragments of this kind are frequent in metaphase-anaphase cells even in such root tips as those in which the resting cells have no fragments. Evidently another mechanism is responsible for the origin of these fragments. Fig. 5 *f*—*k* are nuclei from older parts of the roots treated with concentration 10 for four hours and after one day's recovery (experiment 2). Whereas the nuclei of the meristems were quite destroyed, the nuclei of the elongation zone of the root had distinctly vital structures. The nuclei were often lobated, as shown by the picture. In those cases in which the lobes are two and of similar size (Fig. 5 *g*, *i*) the picture bears a complete resemblance to the illustrations of amitosis which now and then appear in the literature. SAX (1937, p. 220), for instance, pictures amitotic division of the generative pollen nucleus induced by heat treatments. Some observations of the HUSKINS school on the division of prophase-nuclei into two smaller nuclei may be due to similar phenomena.

The disturbances so far described are lethal and irreversible. Ordinarily, roots containing them do not recover. Sometimes, however, recovery has been observed in roots containing fragments of the resting nuclei. In experiment 3 *a*, for instance, in concentration 10, treatment for two hours, there were seen plenty of protuberances and free fragments. On fixing again after four hours' recovery, the roots then studied showed nuclear recovery with seemingly vital structures, and after one day they had an abundance of normal mitoses. Of course, it may be

objected that the same root was studied only once, and that consequently the roots studied on the first occasion may have been the only ones showing lethal changes. Our experience is, however, that the behaviour of all roots is fairly uniform in these treatments.

Above we have given a description of the morphological behaviour of this nuclear reaction without being able to enter on any analysis of its causes. Similar behaviour of the nuclei is, however, also known from other treatments than penicillin. One of us has observed similar behaviour after treatments with light metal salts, as lithium nitrate, potassium, cyanide, *etc.* It should be pointed out that the penicillin employed is a sodium salt, so it is possible that the effect is, partly at least, a salt action. Similar responses are found in lethal treatments with narcotics, for instance, chloroform (STEINEGGER and LEVAN, 1947, p. 518, Figs. 1 *a*—*c*). On treatments with herbicidal chemicals (methoxone, 2, 4-D) DOXEY and RHODES (1949) and NYGREN (1949) have met with similar nuclear fragmentations. The latter author depicts (Pl. 1) numerous instances of so-called apophyses, which even if they are somewhat different in type from our protuberances, certainly are of similar nature. NYGREN writes: »All substances» (*i. e.* 2, 4-D and related derivatives) »caused extruded buds, not only in high concentrations but also in the weakest solutions used, *i. e.* $^1/_{10000}$ mol. The effect was especially obvious for 2-methyl, 4-chlorophenoxyacetic acid, but appeared in this case first 72 hours after the treatment» (time of treatment 2—48 hours). »The nuclei project long apophyses, which are often branched (Fig. 10). The arms of the apophyses break, and in this way fragments are formed» (*l. c.*, p. 726). It seems that the writer in this case considers the reaction to be a vital one and the »bud fragments» arising to be able to survive. In our case it must be pointed out that the reaction is almost regularly combined with complete lethality. We are inclined to consider it due to physical happenings in the cell, not to physiological ones. Judging from the morphology of the reaction, we think that there is involved an increased pressure inside the nucleus, probably caused by osmotic conditions. The nuclear membrane is elastic and resists the pressure at first. Later on, some weak point of the pellicle will yield and bulge out as an extrusion. Chromatic substance gathers in this, and the protuberance may either loosen and form a droplet beside the mother nucleus, or the nuclear membrane may burst on the outer surface of the protuberance and its contents be thereupon thrown out as numerous droplets.

The appearance of this reaction exhibits many resemblances to the

phenomenon of plasmoptysis, in which a young cell-wall bursts at its weakest point, evidently because of a great increase in the pressure inside the cell. Such sudden explosions are common in root-hairs, pollen-tubes and so-called camphor forms of yeast.

The final stage of our »nucleo-plasmoptysis» has the same type as is well-known from degenerative processes in plant and animal tissues, for instance, degenerating endosperm, tumour tissue, *etc.*, although certainly the mechanism of its origin may be different in different cases. Similar pictures have often been described from colchicine treatments of animal material. The original type of DUSTIN's karyoclastic reaction involved not only the checking of the mitosis in concentration stages but also a disintegration of the resting nuclei. This latter effect of colchicine has not been met with in plant material, where even strong dosages (10 %) usually have no morphological effect on the resting nuclei. Similarly, numerous other chemicals which have caused nuclear fragmentations in animals have not been observed to cause such in plant material. Thus, pyrogallol gives rise to nuclear disintegration of the ileac epithelium of mice (AHLSTRÖM and LEVAN, unpublished), while the same type of reaction is not found in the *Allium* test. This differential behaviour towards chemicals between plants and animals is interesting and worthy of attention. However, similarity in morphology between our type of nuclear disintegration described here and other such instances must not be taken as proof of identity of the underlying cytological mechanism. The case here described may possibly have the same mechanism as the most drastic nuclear disintegrations induced by chemicals in animals or very far advanced nuclear degeneration in tumour tissue.

Another toxic cell-reaction, observed in penicillin treatments of lower strength than those treated above, is a pronounced stickiness. This is evident in concentrations 5 and 2, sometimes still further down. The mitotic frequency is strongly depressed or completely inhibited in short treatments with 2 and 1 or in long treatments with 0,5. As is well known, complete absence of mitosis during the early period of a treatment indicates that the chromosomes have ability of despiralizing and entering the resting stage. It is consequently to be regarded as a less severe toxic effect than the heaping up in the tissue of liquefied metaphases and anaphases. Consequently, there is more hope of recovery for a tissue with exclusively resting nuclei than for one showing many liquefied mitoses. Similarly, the ability of the chromosomes to arrange themselves into c-mitosis is an indication of less severe toxic effect than

if the chromosomes remain motionless in their original positions. A certain viability is necessary for the chromosomes to assume the c-mitotic appearance.

II. NARCOTIC CELL REACTIONS.

1. C-TUMOURS.

Penicillin has no pronounced efficiency at inducing the so-called narcotic cell reactions, *i. e.* the c-tumour reaction, an uncontrolled growth of the cells in the elongation region of the root, or the c-mitotic reaction, destruction of the nuclear spindle. A close analysis of the strongest concentrations indicates, however, that both reactions may occur.

C-tumours have never been observed on continuous treatment. Under such treatment toxicity evidently prevails in those concentrations which may give c-tumours. In recovery tests, however, typical c-tumours are met with now and then. They occur in all experiments in the concentration range 1—10. The following data show the minimum dosages which have induced c-tumours.

Concentration	Time of treatment	Time after treatment
1	4 days	1 day
2	4 hours	4 days
5	2 »	2 »
10	2 »	2 »

Shorter treatment with concentrations 1 and 2 than the above-mentioned never gave tumours. Evidently a certain minimum product (concentration × exposure) must be attained.

2. C-MITOSIS.

C-mitosis was not observed in experiment 1, although 24 hours' treatment in concentration 2 induced great chromosome contraction. Experiment 2 showed c-mitosis of ball-type in concentration 10 after two hours' treatment. Four hours' treatment plus two hours in water gave c-mitoses completely destroyed by stickiness. Concentration 5 had c-mitotic tendencies after two hours and normal mitoses again after four hours. The other experiments gave similar results. In no case was concentration 2 able to induce c-mitosis, but 5 gave it rather persistently. In experiment 4 *b*, where the c-mitotic conditions were especially clear, the following data were recorded:

Treatment 4 hours

Concentration	Recovery 4 hours	Recovery 8 hours	Recovery 24 hours
10	sticky ball-mitoses	complete, typical c-mitosis	mitoses inhibited
5	partial c-mitosis, multipolar anaphases	complete, typical c-mitosis	destroyed, sticky c-metaphases, no new divisions
2	exclusively normal mitoses		

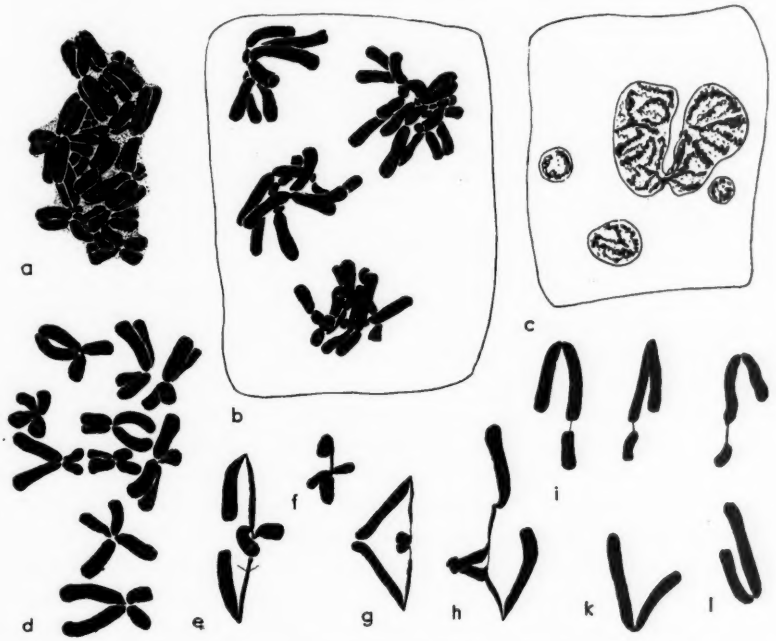


Fig. 6. *a*—*d*: c-mitotic responses to penicillin. *a*: c-metaphase; *b*: multipolar anaphase; *c*: multinuclear telophase; *d*: c-pairs; *e*—*l*: radiomimetic responses; *e*—*h*: pseudochiasmata; *i*—*l*: attached fragments. — × 2000.

In Fig. 6 some pictures from this experimental series are given: *a* is a sticky c-metaphase from concentration 10, the first time of fixation, *b* is a multipolar anaphase, *c* a telophase resulting from such an anaphase, both from concentration 5, *d*, finally, is a complete c-metaphase, quite as characteristic as if it had been induced by colchicine. We have given these data in some detail because we want to point out that under certain conditions penicillin can induce c-mitosis,

although this effect is not always easy to get well-manifested. A strong tendency to liquefaction of the chromosomes tends to make the c-mitosis unclear and the general toxicity of the active concentrations often inhibits the occurrence of mitoses.

The results of c-mitosis agree in all experiments. Its threshold is situated somewhat higher than that of the c-tumour reaction: concentrations 1 and 2, which may give c-tumours, do not induce c-mitosis, while 5 and 10 give complete c-mitosis, most pronounced in the recovery experiments after a short exposure (2—4 hours) and a few hours' recovery.

III. RADIOMIMETIC EFFECTS.

As mentioned in the introduction, the present investigation was originally started in order to control cytologically whether penicillin was a mutagenic substance able to induce radiomimetic effects. Much attention was accordingly directed to the tracing of structural chromosome changes. As a rule, 200 anaphases were scrutinized in each slide, where this was possible. This work led to the detection of many indubitable pseudochiasmata, bridges, attached fragments and even free acentric fragments. The frequency of these abnormalities was, however, always very low. Correlation to the dosage was also difficult to find. In Table 1 a rough summary of the results from all experiments is given. At the heads of the columns the treatment data are given; each filled-in square of the table indicates one analysed slide. 0 means that no signs of any radiomimetic disturbances were found, × indicates the occurrence of other abnormalities than free fragments (*i. e.* attached fragments, pseudochiasmata, *etc.*), • indicates the occurrence of free fragments (their frequency rarely exceeded one or two cases in a single slide).

The table shows that all experiments (except No. 2, in which no fragments were found) had a low frequency of anaphases with acentric fragments, as also other disturbances of radiomimetic type. On comparison with the few controls of the experiments it was found that they never showed any free fragments. Later on, when it was realized that the correlation between dosage and occurrence of free fragments was poor, we were in doubt whether the treatment was responsible for the fragmentation. It was possible that a similar low frequency of spontaneous chromosome breakage had occurred as was recently shown for *Vicia faba* by LEVAN and LOTFY (1950). In order to test this point 20 control bulbs were set up in the autumn of 1950. TJIO analysed 200 anaphases

TABLE 1. *The occurrence of radiomimetic effects.*

Experiment No.		1						3a						3b						4a		4b			
Time of treatment + time of recovery		2 h	4 h	1 d	2 d	4 d + 1 d	4 d + 10 d	2 h	2 h + 1 h	2 h + 4 d	2 h + 4 d	2 h + 12 d	2 h + 21 d	4 h	4 h + 4 h	4 h + 1 d	4 h + 4 d	4 h + 12 d	4 h + 21 d	4 h	1 d	4 h + 4 h	4 h + 8 h	4 h + 1 d	4 h + 4 d
Concentrations	10									0		●	●			0									
	5									0	0	●	0			0				●					
	2	●	×					0	0	0	0					●	●	0	0	×					0
	1		0			×	0							0	0	0	0	0	0	●		×	0	0	×
	0,5		×		0	×	0							×	×	0	0	0	●	●	●	0	×	0	●
	0,2		0	0	×	×	0							0	●	0		0	0	0	0				
	0,1		0	●	×	×								0	0	0	0	0	0	×	0				
	0,05		0	●	×	●								●	×	●	0	0							
	0,02		×	0	×	0												×	0						
	0,01		0	×	×	0																			
	0,005		0	0	0	×																			

from each. In the 4000 anaphases thus studied there occurred no case of free fragment, although a few times a micronucleus in a resting cell was recorded. These may, however, have originated from a vagabonding chromosome, and, therefore, need not necessarily indicate spontaneous fragmentation. This, taken together with our earlier experience of the *Allium Cepa* strain used, makes it fairly certain that the penicillin preparation studied has a certain, although low, radiomimetic activity.

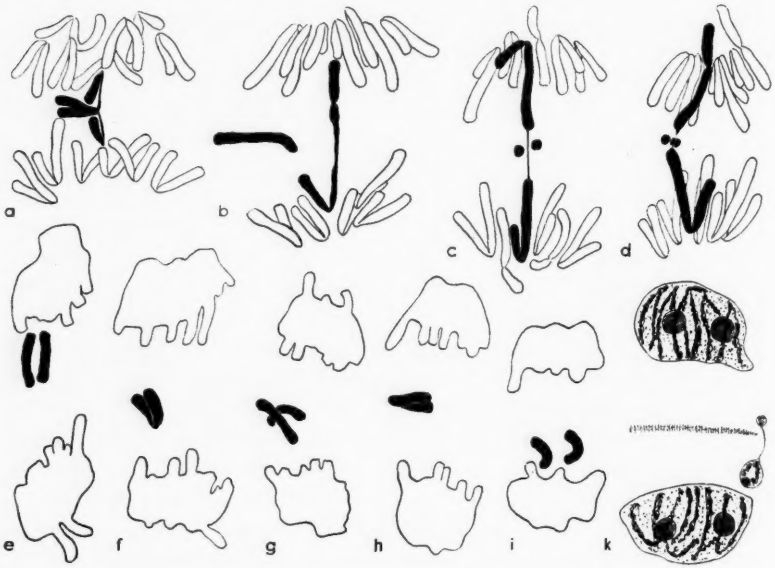


Fig. 7. Radiomimetic responses. *a*: pseudochiasma; *b:* bridge and big fragment; *c*, *d*: pairs of small fragments; *e—i*: telophases with fragments; *e—i*: telophases with micronuclei. — × 1500.

Figs. 6 to 8 show various instances of the observed effects. Pseudochiasmata are pictured in Fig. 6 *e—h* and Fig. 7 *a*. Three cases of attached fragments, or induced secondary constrictions, are represented in Fig. 6 *i—l*. Various cases of free fragments are seen in Fig. 7 *b—i* and Fig. 8. The free fragments are of variable size, from very small ones to big fragments. The fragmentation is of the same type as the spontaneous fragmentation observed by LEVAN and LOTFY (*l. c.*) in *Vicia*, *i. e.* two fragments occur in each case, both of about equal size, indicating that a break within both chromatids of one chromosome is

Fig. 8. Microphotos of various radiomimetic effects. *a*: prophase with micronucleus; *b*: metaphase with free fragment; *c*: anaphase with bridge and fragment; *d*: anaphase with fragment; *e*: telophase with fragment.

responsible for their origin. The proximal part of this chromosome often forms a bridge. There seems to be a transition between pseudochiasma formation and this type of fragmentation. In Fig. 7 *k* a telophase with a couple of micronuclei is shown. Fig. 8, finally, shows microphotos of fragments during various mitotic stages.

SUMMARY.

Allium tests with penicillin showed the following cytological reactions:

(1) Strong treatments (10—20 million international units per 100 cc) induced lethality. All resting nuclei in these treatments were characterized by very typical mushroom-like outgrowths protruding into the plasm. These protuberances often exploded, throwing around chromatic droplets. In extreme cases the entire nucleus might burst into numerous nuclear fragments of varying size.

(2) The c-tumour reaction and c-mitosis were induced only by strong concentrations under very particular conditions. The threshold concentration for the c-tumour reaction was between 0,5 and 1, that for c-mitosis between 2 and 5.

(3) A very low and rather erratic frequency of radiomimetic changes (pseudochiasmata, attached and free fragments, *etc.*) was caused by penicillin, which evidently has some, although very weak, mutagenic activity.

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CYTO-TAXONOMICAL STUDIES IN THE GENUS LUZULA

I. SOMATIC CHROMOSOMES AND CHROMOSOME NUMBERS

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THIS study has been made as an orientation in the taxonomical problems of the genus *Luzula* in relation to its cytology with the object of using the results as a basis for further cyto-taxonomical investigations of the species. To obtain a survey of the cytological conditions in the genus, about forty species belonging to different sections have been examined, their chromosome numbers determined, and their mitotic as well as usually their meiotic chromosomes studied (see Tables 1—5 and Figs. 1—5). About one third of the species investigated are characterized by 12 chromosomes somatically. These chromosomes are always of the same size in the cells. They are 1—2 μ long at metaphase stage with a certain difference in size between species or sections of the genus. They have a uniform thickness of about half a μ and show no constrictions with the fixatives used. This the most common chromosome set will be called the standard or basic chromosome pattern of the genus *Luzula*, and the chromosomes of this pattern will be denoted the AL-chromosome type of the genus *Luzula*. The chromosomes of the other species of the genus are more numerous, with the exception of one single species, *L. purpurea*, which has only six chromosomes somatically.

The chromosome numbers of the species belonging to the genus *Luzula* usually form polyploid series. Some of the high-chromosomal species have chromosomes of about the same size as those of the standard chromosome pattern of closely related species, *i. e.* chromosomes of AL-type. These polyploid species are thus of an ordinary type of polyploidy, in which the species presumably have achieved their high chromosome numbers by doubling or adding up whole chromosome sets of closely related diploids. Other species of the genus with high chromosome numbers have smaller chromosomes than those of the standard chromosome pattern. These species with smaller and more numerous chromosomes are found in various sections of the genus and

are, as polyploids of ordinary type, closely related to species with 12 chromosomes of standard pattern. Moreover, there seems to be a certain relationship between the number of chromosomes and their size in the different species. When the number of chromosomes is doubled from 12 to 24 the length of the chromosomes is reduced to about half that of the standard length (compare *L. glabrata* and *L. spadicea* with *L. parviflora* and *L. Wahlenbergii* of Fig. 2, and see *L. spicata*, Fig. 4 *a—c*). This smaller chromosome type has been called the BL-type. Some species with 48 chromosomes have a chromosome size of about half that of the BL-type and they are said to have the CL-type of *Luzula* chromosomes (see *L. sudetica*, Fig. 3 *g*, and compare *L. Forsteri* and *L. luzulina* with *L. acuminata* of Fig. 5). These chromosomes of CL-type are almost spherical at metaphase stage. Further reduction of the chromosome size is met with in connection with increased chromosome number (see *L. pilosa*, Fig. 5 *f*), but here the chromosomes are too small to allow of any detailed examination. The majority of the species here investigated have, as described, about the same chromosome size throughout in the cells. In some cases, however, distinct differences in chromosome size are found in the cells of a species. This phenomenon is often connected with an aneuploid chromosome number. In the chromosome pattern of the aneuploids of this kind two chromsomes of BL-type seem to have replaced one of AL-type of the standard chromosome pattern, and two chromosomes of CL-type appear to correspond to one of BL-type in a small-chromosomal polyploid (see *L. spicata*, Fig. 4 *a—c*, and compare the diploids of the *campestris-multiflora* complex with *L. orestra* of Fig. 3). This given relation between the chromosome types seems to occur in all cases where it could possibly be checked. As an example of this type of polyploidy we can mention one of the chromosome races of *L. spicata* with 14 chromosomes, 10 of AL-type and four of BL-type. The two other chromosome races of that species have 12 chromosomes of AL-type and 24 chromosomes of BL-type (see Fig. 4 *a—c*).

Only one species is studied in which the chromosomes are larger than about 2 μ. This species is *L. purpurea* with six chromosomes somatically. The chromosome length is here about 4—6 μ.

THE CHROMOSOME PATTERN IN RELATION TO SYSTEMATIC ARRANGEMENTS.

As regards the systematic arrangements in the genus *Luzula*, I have principally followed that of BUCHENAU (1890, 1906). In the names of

the species there exists great confusion in many instances. This is especially the case with the *campestris-multiflora* complex. This complex comprises a large number of closely related taxonomical units, many of which have been described as independent species, others as varieties or subspecies under different species. It seems not yet possible for me to settle definitely the question of their taxonomical rank. For that reason, I have for the present preferred to mention them by the names most commonly used for them in the recent literature, sometimes using the names of local races for species or varieties in their whole distribution. On the other hand, I have not searched for the earliest published designation. A more thorough morphological description of the species and races in relation to their taxonomy will be given later together with the results of culture studies and hybridization experiments.

According to BUCHENAU's systematic arrangements in the genus *Luzula*, this genus is divided into three subgenera: *Anthelaea*, *Gymnodes*, and *Pterodes*. We will treat them in the order given above so as to have in the first place a description of the simpler types of chromosomal variation. In this way we shall get some understanding of the diverse ways of chromosomal differentiation which have taken place in the different sections of the genus.

ANTHELAEA.

The subgenus *Anthelaea* represents the section of the genus *Luzula* with the simplest chromosome patterns. The species of this subgenus can be separated into three groups. The first contains only one single species, *L. purpurea* LINK, which is most widely separated from all other species of the genus. It is also cytologically different from all species of the genus *Luzula* which have been cytologically examined. It has only six chromosomes somatically, which at metaphase are about 4—6 μ long and about one μ thick (see Fig. 1). This size is much larger than that of the chromosomes of any other species examined in this genus. The material studied here originates from the Botanical Gardens of the University of Coimbra in Portugal. It is thus the same material as was studied earlier by CÂMARA and coworkers (MALHEIROS, DE CASTRO and CÂMARA, 1947; MALHEIROS and DE CASTRO, 1947; and DE CASTRO, CÂMARA and MALHEIROS, 1949).

The two other groups of the subgenus *Anthelaea* we may call the *silvatica* and the *parviflora* groups, taking the names of their most widespread species as denomination for the groups. The chromosome pattern

for the *silvatica* group seems to be entirely uniform. All the species investigated have 12 chromosomes of the standard or AL-type, and five out of eleven species known have been studied (see Table 1 and Fig. 2 *a*—*e*). The results of earlier examinations of *L. silvatica* (HUDS.) GAUD. (WULFF, 1939; NORDENSKIÖLD, 1949; and DE NORONHA WAGNER, 1949),

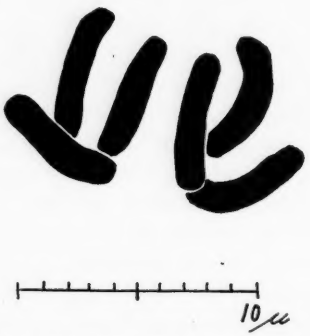

Fig. 1. Camera lucida drawing of a somatic metaphase plate of *L. purpurea*.

L. Henriquezii DEGEN (MALHEIROS and GARDÉ, 1947), *L. luzuloides* (LAM.) DANDY and WILM. (NORDENSKIÖLD, 1949 and DE NORONHA WAGNER, 1949), *L. lutea* (ALL.) DC. (DE NORONHA WAGNER, 1949), and *L. nivea* LAM. and DC. (NORDENSKIÖLD, 1949 and DE NORONHA WAGNER, 1949) have been verified. Other species of the group, as *L. lactea* LINK

TABLE 1. *Species, original localities, and chromosome numbers of*

Species	Localities
L. silvatica	Bot. Gardens of the Univ., Uppsala
»	» » » » » Göttingen
»	Polster, Präbichl, Styria
L. Henriquezii	Bot. Gardens of Gothenburg
L. lutea	» » » »
L. luzuloides	Freiburg im Br., Schwarzwald, Germany
»	Fischbach, Styria
»	Maria Trost, near Graz, Styria
»	Glashütten, Koralpe, Styria
»	Polster, Präbichl, Styria
»	Patscherkofel, Tyrol
»	Stubalpe, Styria
L. nivea	Bot. Gardens of Univ., Uppsala
»	Vaglio, Tesserete, Kt. Tessin, Switzerland

and *L. Seubertii* LOWE, have also been found to have 12 chromosomes somatically (MALHEIROS and GARDÉ, 1947). All these species are from the mountain regions of south and south-western Europe, though *L. silvatica* and *L. luzuloides* have a more widespread distribution. Thus, many of the species occupy the same geographical area and the whole group is restricted to a rather small region.

In the *parviflora* group of the subgenus *Anthelaea* different types of chromosome patterns have been found in the species investigated up to date. *L. glabrata* DESV. and *L. spadicea* (ALL.) DC., the two alpine species of the south European mountains of this group, have 12 chromosomes of standard or AL-type. *L. parviflora* (EHRH.) DESV. and *L. Wahlenbergii* RUPR., the subalpine and alpine species of the arctic regions of this group, and *L. subcongesta* JEPS., an alpine species from Sierra Nevada in California, have, on the other hand, 24 chromosomes of BL-type (see Table 2 and Fig. 2 *f*—*j*). The chromosome number of *L. parviflora* here shows agreement with chromosome counts earlier made by LÖVE and LÖVE (1944), NORDENSKIÖLD (1949) and BÖCHER and LARSEN (1950), and the same is the case with *L. spadicea* as determined by DE NORONHA WAGNER (1949). The chromosome number of *L. Wahlenbergii* given by LÖVE and LÖVE (1944) is $2n = 36$. This count does not agree with the one found by the author in the present investigation. Probably the results of LÖVE and LÖVE cannot be considered correct. From their drawing of the chromosomes of this species (p. 3,

the material studied in the subgenus Anthelaea (silvatica group).

Habitat	Collector	Material collected	Numb. of chromosome determined plants	$2n$
		1 pl	1	12
		seeds	3	12
Hillside in dry open wood	Author	1 pl	1	12
		1 »	1	12
		1 »	1	12
	G. TURESSON	1 »	1	12
Dry open woodland	Author	6 »	3	12
» » »	»	7 »	3	12
» » »	»	4 »	1	12
» hillside at 1800 m. alt.	»	5 »	2	12
» » » » » »	»	9 »	6	12
1500—1600 m. alt.	F. WIDDER 13	6 »	1	12
		1 »	1	12
Chestnut wood at 580 m. alt.	W. KOCK 50/93	2 »	2	12

TABLE 2. *Species, original localities, and chromosome numbers of*

Species	Localities
L. parviflora	Vassijaure, Lapland, Sweden
»	Mt. Washington, N. H., U. S. A.
»	» » » »
»	» » » »
»	» Albert, Que., Canada
»	Compton Co., Que., Canada
»	White Horse, Yukon, Canada
»	9 miles S. Anchorage, Alaska
»	Lake Louise, Alta., Canada
»	Tillamook Co., Oregon, U. S. A.
»	Rock Creek, Tulare Co., Calif., U. S. A.
L. glabrata	Reiteralpe, Bayerische Alpen, Germany
»	Polster, Präbichl. Styria
»	Eisenerzen Alpen, Styria
L. Wahlenbergii	Nissonreppe, Lapland, Sweden
»	Nissontjorro, » »
»	Vassijaure, » »
L. spadicea	Bot. Gardens of Gothenburg
»	Hohe Tauern, Glocknergruppe, Austria
»	Patscherkofel, Tyrol
»	Geisskogl bei Kühtai, Tyrol
»	Stubalpe, Styria
»	» »
L. subcongesta	Rock Creek, Tulare Co., Calif., U. S. A.

Fig. 6, *l. c.*) one gets the impression that the determination has been made on a specimen of *L. arcuata*, the characteristic chromosome pattern of which is given in the picture. The material for their study originates from Mt. Låktatjokko in northern Lapland, where *L. arcuata* is a very common species, in contrast to *L. Wahlenbergii*. All the species of the *parviflora* group are very closely related and many of them hard to distinguish. They are distributed all over the mountain regions of the Northern hemisphere, as well as in the arctic regions. The species of the group usually occupy different areas, and if more than one is found in the same geographical region the species grow ecologically separated. The alpine species of the European mountains, *L. spadicea*, and the arctic species of the North, *L. Wahlenbergii*, are morphologically similar

the material studied in the subgenus Anthelaea (parviflora group).

Habitat	Collector	Material collected	Numb. of chromosome determined plants	2*n*
Gravel ground at abt. 1500 m. alt.	A. NYGREN	seeds	5	24
Moist meadow-like ground, 1600 m. alt.	Author	4 pl	4	24
	»	seeds	3	24
Along brook at 1400 m. alt.	»	»	3	24
Moist open wood at 800 m. alt.	W. G. DORE 47—782	»	3	24
» side at 2500 ft. alt.	J. A. CALDER 1099	»	1	24
Spruce wood	J. M. GILLETT 4594	»	3	24
	DUTILLY, LE PAGE and O'NEILL	»	2	24
Open wood at 1750 m. alt.	Author	»	4	24
	D. D. KECK	»	3	24
At 9500—10000 ft. alt.	J. T. HOWELL 26127	»	3	24
	Mainz Bot. Gard.	»	3	12
Dry mountain slope at abt. 2000 m. alt.	Author	13 pl	3	12
At 2100 m. alt.	F. WIDDER 10	8 »	3	12
» 1200—1300 m. alt.	A. NYGREN	6 »	5	24
Wet alpine meadow at 1100 m. alt.	O. HEDBERG	4 »	3	24
	A. NYGREN	6 »	5	24
		1 »	1	12
At 2400 m.	F. WIDDER 8	1 »	1	12
Along brook at 1750 m. alt.	Author	3 »	2	12
» » » 2100 » »	»	9 »	3	12
At 1990 m. alt.	F. WIDDER 11	5 »	1	12
» 1800 » »	» 14	4 »	4	12
» 10500 ft. alt.	J. T. HOWELL 26216 A	seeds	5	24

in spite of their difference in chromosome numbers, and the same is the case with the two other corresponding species of the same regions, *L. glabrata* and *L. parviflora.*

GYMNODES.

Gymnodes is the largest subgenus of the genus *Luzula.* Many of the species of that subgenus belong, however, to the Southern hemisphere, especially to South America and New Zealand. In these parts of the world neither of the two other subgenera, *Anthelaea* or *Pterodes*, are represented. Species of the Southern hemisphere have, however, not been dealt with in this study. Furthermore, the species studied here are from only three sections of this large subgenus. These three sections will be

named the *spicata* group, the *arctica* group, and the *campestris-multiflora* complex.

Among the species belonging to the *spicata* group only *L. spicata*

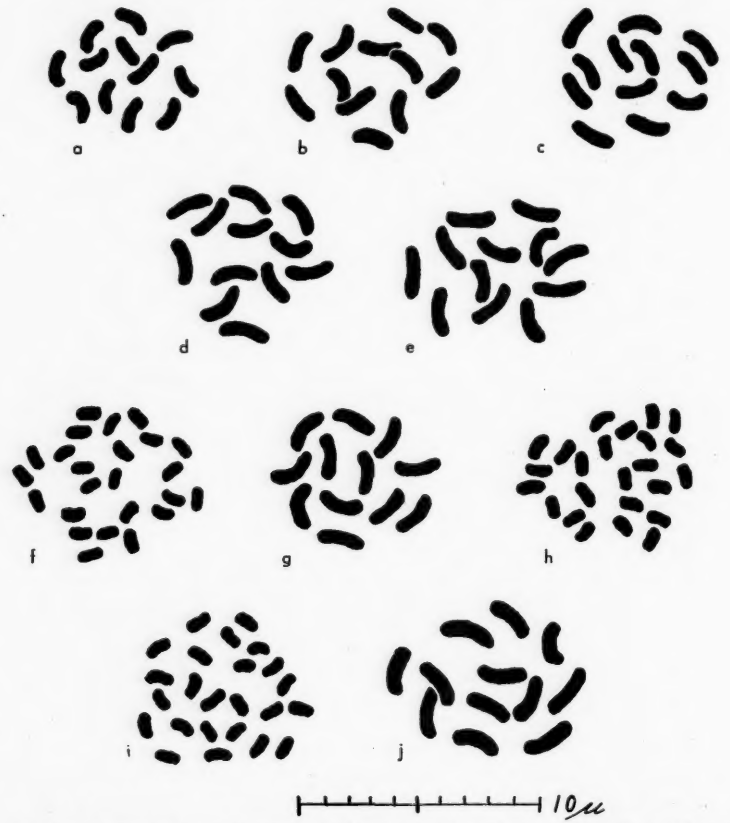

Fig. 2. Somatic metaphases of species belonging to the subgenus *Anthelaea*. (Camera lucida drawings.) (*a*) *L. silvatica* $2n = 12$; (*b*) *L. Henriquezii* $2n = 12$; (*c*) *L. lutea* $2n = 12$; (*d*) *L. luzuloides* $2n = 12$; (*e*) *L. nivea* $2n = 12$; (*f*) *L. parviflora* $2n = 24$; (*g*) *L. glabrata* $2n = 12$; (*h*) *L. subcongesta* $2n = 24$; (*i*) *L. Wahlenbergii* $2n = 24$; (*j*) *L. spadicea* $2n = 12$.

(L.) DC. and *L. abyssinica* PARL. have been studied (see Table 3). The former species has a very wide distribution over the whole arctic and mountain regions of the Northern hemisphere. The most common chromosome pattern of this species is 24 chromosomes of BL-type. This

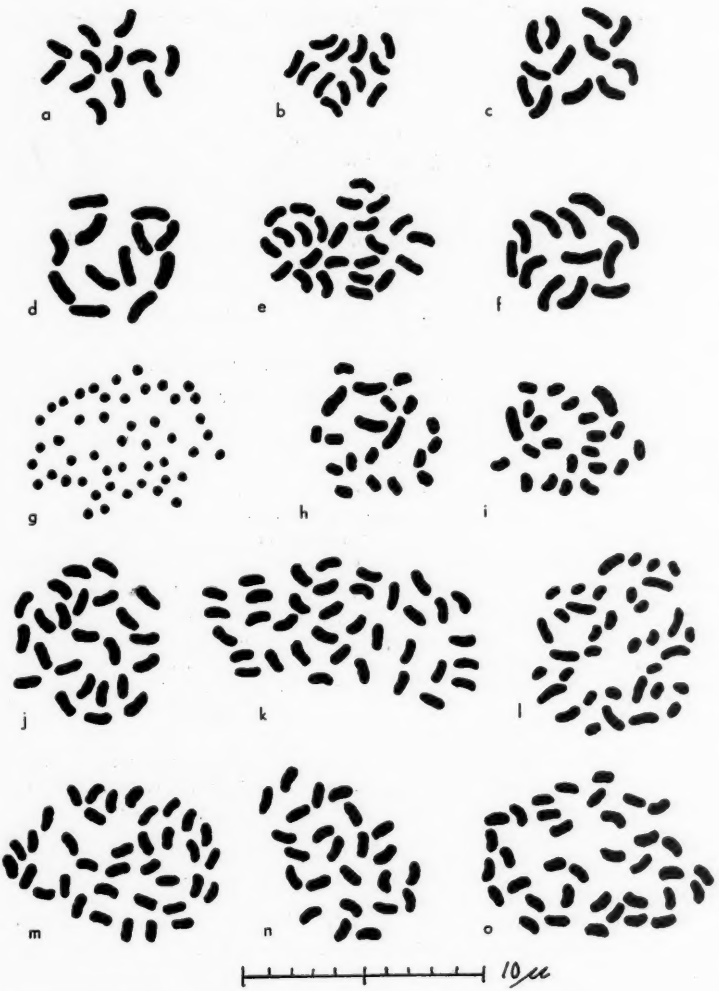

Fig. 3. Different chromosome patterns of the species and races belonging to the *campestris-multiflora* complex. Camera lucida drawings of somatic metaphases. (*a*) *L. campestris s. str.* $2n = 12$; (*b*) *L. pallescens* $2n = 12$; (*c*) *L. echinata* $2n = 12$; (*d*) *L. comosa* v. *macrantha* $2n = 12$; (*e*) *L. comosa s. str.* $2n = 24$; (*f*) *L. comosa* v. *laxa* $2n = 12$; (*g*) *L. sudetica* $2n = 48$; (*h—i*) *L. orestra* $2n = 20$ and 22; (*j*) *L. groenlandica* $2n = 24$; (*k*) *L. frigida* $2n = 36$; (*l*) *L. campestris* v. *vallesiaca* $2n = 36$; (*m—n*) *L. multiflora* $2n = 36$ and 24; (*o*) *L. congesta* $2n = 36$.

TABLE 3. *Species, original localities, and chromosome numbers of the*

Species	Localities
L. spicata	Vassijaure, Lapland, Sweden
»	Helags, Härjedalen, »
»	Routats, Lapland, »
»	Frostviken, Jämtland, »
»	Storsnasen, » »
»	Karesuando, Gunnarin Korva, Lapland, Sweden
»	Flatruet, Härjedalen, Sweden
»	Mt. Washington, N. H., U. S. A.
»	Army Pass, Inyo Co., Calif., U. S. A.
»	Koralpe, Hauptkamm, Styria
»	Hohe Tauern, Glocknergruppe, Austria
»	Patscherkofel, Tyrol
»	Stockacher Bach, Tyrol
»	Moschkogl, Koralpe, Styria
»	Koralpe, Hauptkamm, »
»	Stubalpe, Styria
L. abyssinica	Mt. Kenya, Teleki Valley, Central Africa
»	Kilimanjaro, near Peter's Hut, Central Africa
L. arctica	Päldsa, Lapland, Sweden
»	Nissonreppe, Lapland, Sweden
L. arcuata coll.	Helags, Härjedalen, Sweden
»	Nissonreppe, Lapland, Sweden
»	» » »
»	Baffins Island, Coutts Inlet, 72°15′ N; 74°30′ W
»	Melville Peninsula, Committee Bay, 68°18′ N; 85°25′ W
»	E. Greenland, Angmagssalik, 65°40′ N

pattern is found in plants studied from North America, *i. e.* Sierra Nevada in California as well as Mt. Washington in New England. Counts given from Greenland (BÖCHER, 1938; SÖRENSEN and WESTERGAARD in LÖVE and LÖVE, 1948) have also yielded the same chromosome number, $2n = 24$. In Europe this same chromosome pattern is found in the Scandinavian mountains and in the Tyrol in the Alps. East of this region, however, in the Ostalpen, different chromosome races are found. In the Koralpe and Saualpe, the most south-eastern offset of the Alps, the chromosome number of the species is 14, with 10 chromosomes of AL-type and four of BL-type. One sample from the Gross Glockner massif of Hohe Tauern collected by Dr. F. WIDDER has the chromosome

material studied in the subgenus Gymnodes (spicata and arctica groups).

Habitat	Collector	Material collected	Numb. of chromosome determined plants	2*n*
	A. NYGREN	seeds	4	24
At abt. 1100 m. alt.	Author	5 pl	2	24
Serpentine soil	S. O. BJÖRKMAN 2859	1 »	1	24
	»	seeds	2	24
At 1400 m. alt.	B. LÖVKVIST	8 pl	2	24
	S. O. BJÖRKMAN 3030	1 »	1	24
	G. TURESSON	10 »	2	24
Rocky ground at 1700—1900 m. alt.	Author	8 »	6	24
At 12000 ft. alt.	J. T. HOWELL 26059	seeds	1	24
Rocky ground at 1900—2000 m. alt.	F. WIDDER 4	1 pl	1	14
At 2400 m. alt.	» 9	1 »	1	12
» 1900 » »	Author	15 »	5	24
Alpine meadow at 1800 m. alt.	»	14 »	7	24
Dry hillside at 1700 m. alt.	»	13 »	6	14
At abt. 2000 m. alt.	»	14 »	3	14
» 1600 m. alt.	F. WIDDER 12	4 »	3	14
» 4050 » »	O. HEDBERG 1766	seeds	2	24
» 3650 » »	» 1216	»	1	24
	A. NYGREN	5 pl	2	24
	»	20 »	2	24
» 1200 » »	Author	25 »	4	36
» abt. 1000 m. alt.	A. NYGREN	10 »	8	42
» » » » »	»		2	36
	D. COOMBS 89	seeds	2	36
Moist, sandy beach	J. B. WOODRUFF	»	3	36
	J. LAGERKRANZ	»	2	36

number $2n = 12$, with all the chromosomes of AL-type (see Fig. 4 *a*—*c*). The second species of this group which has been examined is *L. abyssinica*. Two samples, one from Mt. Kenya and one from Mt. Kilimanjaro in Central Africa, collected by Dr. O. HEDBERG, were studied. Both samples had 24 chromosomes somatically, all of BL-type (see Fig. 4 *d*). That is the same chromosome pattern as the most common one of *L. spicata*.

The second group studied in the subgenus *Gymnodes* is the *campestris-multiflora* complex. Geographically it is the most widespread section of the genus occupying the arctic, temperate, and mountain regions of both Northern and Southern hemispheres. (All the species

TABLE 4. *Species, original localities, and chromosome numbers*

Species	Localities
L. campestris s. str.	Kila, Södermanland, Sweden
»	Uppsala, Uppland, »
»	Steninge, » »
»	Hjälstavik, » »
»	Alingsås, Västergötland, »
»	Hindås, » »
»	Leeds, England
L. pallescens	Ånge, Medelpad, Sweden
»	Åsarna, Jämtland, »
»	Kila, Södermanland, »
»	Fischbach, Styria
»	Kufstein, Niederried, Tyrol
L. echinata	Delaware Co., Penna., U. S. A.
»	Washington, D. C., U. S. A.
L. bulbosa	Farnham, Missisquoi Co., Que., Canada
L. comosa v. *macrantha*	Berkeley, Calif., U. S. A.
»	Stanford, » »
»	Monterey Co., Calif., »
»	Marin Co., Calif., »
»	Vancouver Isl., B. C., Canada
L. comosa v. *laxa*	Ackerson Creek, Tuolumne Co., Calif., U. S. A.
»	Harrison Springs, B. C., Canada
»	Vancouver Isl., B. C., »
L. campestris v. *columbiana*	Mono Co., Calif., U. S. A.
»	Rock Creek, Tulare Co., Calif., U. S. A.
L. sudetica	W. of Ljungdalen, Härjedalen, Sweden
»	Between Ljungdalen and Kesudalen, Härjedalen, Sweden
»	Kesudalen, Härjedalen, Sweden
»	Helags, » »
»	Enafors, Jämtland, »
»	Bergfors, E. Abisko, Lapland, »
»	Koralpe, Styria
»	Seekar, Koralpe, Styria
»	Stockacher Alm, Tyrol
»	Witmoos, Sörenberg, Kt. Luzern, Switzerland
L. orestra	Tuolumne Co., Calif., U. S. A.
»	Cottonwood Lake, Inyo Co., Calif., U. S. A.
»	W. side Army Pass, Tulare Co., Calif., »
»	Spuller Lake, Mono Co., Calif., »
L. groenlandica	James Bay, 45°20′ N, Ont., Canada
L. campestris v. *vallesiaca*	Patscherkofel, Tyrol

of the material studied in the campestris-multiflora complex.

Habitat	Collector	Material collected	Numb. of chromosome determined plants	$2n$
Drier parts of meadow	Author	14 pl	11	12
Moraine hillock	»	21 »	8	12
» »	»	6 »	4	12
Dry meadow	»	30 »	13	12
Moraine hillock	»	6 »	3	12
Dry meadow	»	15 »	8	12
	H. G. BAKER	6 »	4	12
Ditch along wood path	Author	3 »	3	12
Moist open woodland	»	8 »	8	12
Path through wood	»	10 »	3	12
Open woods	»	3 »	3	12
At 600 m. alt.	F. PRENN	seeds	2	12
In rich woods	G. L. STEBBINS JR.	3 pl	2	12
Along brook through wood	Author	2 »	2	12
	M. RAYMOND	seeds	2	12
Hillside along shaded path	Author	30 pl	11	12
» under oaks	»	21 »	6	12
Open oak woods	D. D. KECK	10 »	3	12
In pine woods	J. T. HOWELL 25317	seeds	3	12
At edge of wood along beach	Author	»	3	12
Along riverlet through open wood	»	»	3	12
Moist wood	»	»	5	12
At edge of wood	»	»	7	12
	C. W. SHARSMITH 5700	»	3	12
» 10500 ft. alt.	J. T. HOWELL 26215 A	»	3	12
Moist meadow	Author	36 pl	22	48
» » near brook abt. 800 m. alt.	»	12 »	9	48
Bog at 900 m. alt.	»	7 »	7	48
Moist meadow at 1000 m. alt.	»	3 »	3	48
	O. HEDBERG	1 »	1	48
	A. NYGREN	10 »	4	48
On mountain ridge at 2000 m. alt.	Author	1 »	1	48
In fen at 1900 m. alt.	»	12 »	6	48
» » » 2000 » »	»	2 »	2	48
At 1225 m. alt.	I. AREGGER	6 »	4	48
	C. W. SHARSMITH 5803	seeds	6	22
» 11100 ft. alt.	J. T. HOWELL 26245 A	»	5	20
» 11500 » »	» 26026	»	5	20
» 10420 » »	D. D. KECK	»	2	22
Peaty slope		»	6	24
Open, dry wood at 1300—1500 m. alt.	Author	5 pl	4	36

Species	Localities
L. campestris v. *vallesiaca*	Patscherkofel, Tyrol
»	Stockacher Alm, »
L. comosa s. str.	Mather, Tuolumne Co., Calif., U. S. A.
»	Hetch-Hetchy, Tuolumne Co., Calif., »
»	Bear Creek, Mariposa Co., Calif., »
»	Big Creek, Tuolumne Co., Calif., »
»	Vancouver Isl., B. C., Canada
L. multiflora	Kila, Södermanland, Sweden
»	Uppsala, Uppland, »
»	Ånge, Medelpad, »
»	Åsarna, Jämtland, »
»	Ljungdalen, Härjedalen, »
»	Hindås, Västergötland, »
»	Stenkyrka, Gotland, »
»	Lummalunds bruk, Gotland, »
»	Kappelshamn, Gotland, »
»	Holmhälla, Gotland, »
»	Hejnum, Gotland, »
»	Near Ekeby, Gotland, »
»	» » »
»	Vaglio, Tersserte, Kt. Tessin, Switzerland
»	Normandie, France
»	Fischbach, Styria
»	Glashütten, »
»	» »
»	Carleton Co., Ont., Canada
»	Granton, Pictou Co., N. S., »
»	Lynn, Colchester Co., N. S., »
»	Caraquet, Gloucester Co., N. S., »
»	Boulton, Que., »
»	Cypress Hills Park, Sask., »
»	Compton Co., Que., »
»	Franklin Co., Vermont, U. S. A.
»	Caledonia » » »
»	Bretton Woods, N. H., »
»	» » » »
»	» » » »
»	» » » »
»	» » » »

Habitat	Collector	Material collected	Numb. of chromosome determined plants	2n
At 1700—1900 m. alt.	Author	36 »	26	36
Dry hillside at 2000 m. alt.	»	9 »	6	36
Open moist wood, 1400 m. alt.	»	seeds	8	24
Moist meadow ground	»	»	2	24
Along brook through open land	»	»	4	24
» » » wood	»	»	3	24
At edge of wood	»	»	6	24
Meadow ground	»	13 pl	6	36
» »	»	28 »	18	36
Ditch along wood path	»	15 »	13	36
Moist open woodland	»	3 »	3	36
Bank of a river	»	21 »	21	36
In various more or less wooded bogs.	»	50 »	8	36
	B. Pettersson	7 »	5	36
Open dry pine wood	Author	20 »	10	36
Salty marsh	»	15 »	4	36
Edge of wood	»	12 »	2	36
Wood	»	2 »	2	36
Open dry pine wood	»	12 »	4	36
Bog	»	5 »	5	36
Chestnut wood, at 580 m. alt.	W. Kock 50/92	2 »	1	36
	Rouen Bot. Gard.	seeds	2	36
Open woodland	Author	8 pl	8	36
Along path in wood at 1200—1300 m. alt.	»	25 »	15	36
Along path in wood at 1200—1300 m. alt.	»		8	24
Field, sandy soil	H. J. Soper, J. A. Calder and J. M. Gillett 3083	seeds	1	36
Open woods	W. G. Dore and E. Gorham 45434	»	1	36
Shaded ravine among rocks	W. G. Dore 451096	»	2	36
Old hay field	W. G. Dore and E. Gorham 45652	»	1	36
Wet meadow	J. A. Calder 1211	»	2	36
Wooded banks at 4000 ft. alt.	A. J. Breitung 4580	»	3	36
Dry spruce-maple woods, in humus-rich soil	J. A. Calder 1145	»	5	24
	R. T. Clausen	»	2	24
	»	»	2	24
Road side in wood	Author	13 pl	6	36
» » » »	»		6	24
Bog	»	8 »	2	36
»	»		2	24
»	»		4	30

Species	Localities
L. multiflora	Oze, Gumma Prefecture, Japan
L. frigida	W. Ljungdalen, Härjedalen, Sweden
»	Between Ljungdalen and Kesudalen, Härjedalen, Sweden
»	Helags, Härjedalen, Sweden
»	Abisko, Lapland, »
»	Karesuando at Gunnarin Korva, Lapland, Sweden
L. congesta	Hisingen, near Gothenburg, Sweden
»	» » » »

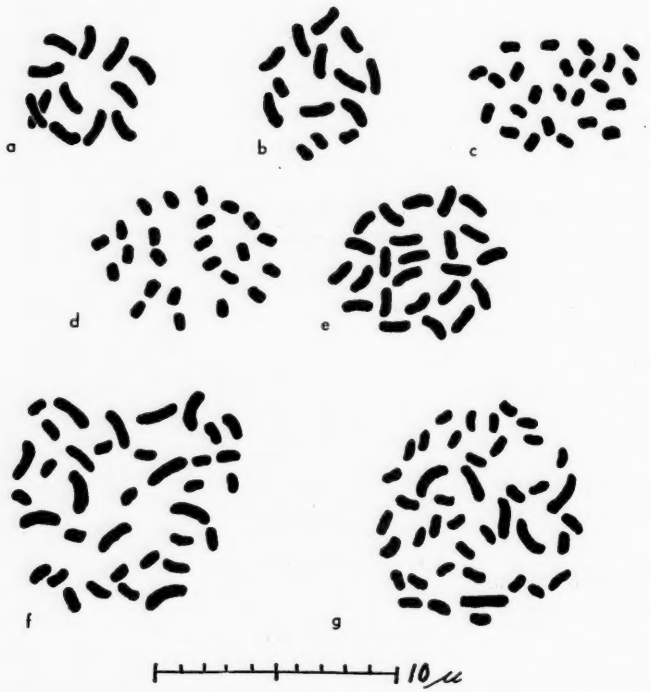

Fig. 4. Somatic metaphases of species and races belonging to the *spicata* and *arctica* groups of the subgenus *Gymnodes*. (Camera lucida drawings.) (*a*—*c*) *L. spicata* $2n = 12$, 14, and 24; (*d*) *L. abyssinica* $2n = 24$; (*e*) *L. arctica* $2n = 24$; (*f*—*g*) *L. arcuata coll.* $2n = 36$ and 42.

Habitat	Collector	Material collected	Numb. of chromosome determined plants	$2n$
	M. FUJITA	seeds	2	24
Moist meadow	Author	5 pl	5	36
Sandy edge at path at abt. 750 m. alt.	»	13 »	9	36
Moist meadow at abt. 1000 m. alt.	»	13 »	12	36
Willow region at abt. 900 m. alt.	A. NYGREN	seeds	5	36
	S. O. BJÖRKMAN 3031	1 pl	1	36
Dry, sparsely wooded bog	Author	11 »	7	48
» » » »	»		2	36

studied belong, however, to the Northern hemisphere.) This section of the genus is composed of a very variable complex of closely related species and races. The chromosomal variation is also much more complicated than that of the earlier described sections. Nevertheless, about one third of the taxonomical units investigated in the *campestris-multiflora* complex have the standard type of chromosome pattern, *i. e.* 12 chromosomes of AL-type (see Table 4 and Fig. 3). The other units examined have chromosome patterns which in different ways seem to be derived from the standard type. Three different kinds of chromosomal deviation can be traced in the complex: First we have an ordinary type of polyploidy, in which the standard chromosome set is doubled twice, three or four times to tetraploids, hexaploids or octoploids. As the second kind of chromosomal variation we have the type of polyploidy, described above, in which the chromosome size is reduced when the chromosome number is raised. The third and last type of chromosomal variation is the kind of aneuploidy described earlier, in which different types of chromosomes are found in the same chromosome set.

The species of the *campestris-multiflora* complex that have been studied here are demonstrated in Table 4 with their localities, chromosome numbers, *etc.* The diploids of the complex usually have a geographically separated distribution. Sometimes, however, two of them occupy the same geographical area, as, for instance, *L. campestris* (L.) DC. (in restricted sense) and *L. pallescens* SW. in Scandinavia or *L. comosa* E. MEYER v. *macrantha* WATS. and *L. comosa* E. MEYER v. *laxa* BUCHENAU (1906) in western North America, but in both places they are ecologically separated. In the non-alpine parts of western North America we have, as it seems, three species or varieties of the complex with broad leaves and big flowers, which morphologically show a rather

marked similarity. Two of these, *L. comosa* v. *macrantha* and *L. comosa* v. *laxa*, are diploids and grow ecologically separated; the former in dry open regions not too far from the coast and the latter in moist open woods. In about the same habitat as v. *laxa* we have *L. comosa* (MEYER) WATS. (*s. str.*), which, however, is tetraploid. The only area where they all three were collected in each other's vicinity was on Vancouver Island. At this place they all grew ecologically separated, and were morphologically distinct.

L. sudetica (WILLD.) DC. has been found to have 48 chromosomes of CL-type (see Fig. 3 *g*), the same number as was earlier found (NORDENSKIÖLD, 1949). It is rather common in the Scandinavian mountains and in bogs southwards down to Middle Sweden, but it is also found in the high wet places in the Alps. *L. orestra* SHARSMITH *ined.* from the Sierra Nevada in California has been found in two different chromosome races with 20 and 22 chromosomes (see Table 4 and Fig. 3 *h*—*i*). The former race from south Sierra Nevada has four chromosomes of AL-type and 16 of BL-type, the latter from more northern latitudes has two of AL-type and 20 of BL-type. *L. sudetica* and *L. orestra* are morphologically very similar and are probably to be regarded as related to each other.

Both *L. sudetica* and *L. orestra* must be regarded as distinct from *L. groenlandica* BÖCHER with $2n=24$ (BÖCHER, 1950). The latter species is a tetraploid of ordinary type with all its chromosomes of AL-type (see Fig. 3 *j*). It is found in Greenland (BÖCHER and LARSEN, 1950, and BÖCHER, 1950) and in Northern Canada (see Table 4 and BÖCHER, 1950). Another alpine race, which has some similarities with *L. groenlandica*, is described from the western parts of the Alps as *L. campestris* (L.) DC. v. *vallesiaca* BEAUVERD (1918). It is shown to hold $2n = 36$, having 12 chromosomes of AL-type and 24 of BL-type. It has, thus, a chromosome pattern built up from one set of standard type added to one set of 24 chromosomes of BL-type (see Fig. 3 *l*). It is found in the drier regions of the mountains. In the higher regions it may occur in the vicinity of *L. sudetica*. In a locality near Kühtai in the Tyrol at 2000 m. altitude the dry hillside was occupied by *L. campestris* v. *vallesiaca* while *L. sudetica* occurred in the bog below at a distance of only a few metres.

L. multiflora (RETZ.) LEJ. (in restricted sense) with $2n = 36$ is a real polyploid of ordinary type with all its chromosomes of AL-type (see Fig. 3 *m*). It has probably the widest distribution of any species in the *campestris-multiflora* complex. It is known from the whole of Europe and the north-eastern part of temperate North America, west-

wards as far as to the Rocky Mountains. It is a very variable species, and some of its races have been taxonomically distinguished. Among these are *L. frigida* (*sensu* SAMUELSSON), the alpine and arctic *multiflora* race of the Scandinavian mountains, Greenland, and eastern North America. Another race, *L. congesta* (THUILL.) LEJ., the congested, Atlantic type of the species, is found in western Europe. I have, however, had material from only one locality, Hisingen, near Gothenburg. Among the investigated plants of that material only two were found to have $2n = 36$, seven, on the other hand, were found to have $2n = 48$, with all chromosomes of AL-type. If it really will be possible to find any special difference between the octoploids and the hexaploids among the congested types of *L. multiflora* cannot be settled from only one sample. For that reason I will, for the present, keep the name *L. congesta* for the congested type of both these polyploids, as that will best correspond to the use of the name in the recent literature. In the southern parts of the distribution regions of *L. multiflora* we find another representative of that species, *i. e.* a race with 24 chromosomes with all its chromosomes of AL-type (see Fig. 3 *n*). It is found in the mountain regions of the northern Appalachian Mountains in New England and in corresponding regions of the Ostalpen in Austria. In the latter region it was also found rather high up in the mountains (in the Koralpe at 1700 m. altitude) and there it forms a dark-flowered alpine race. A tetraploid race of *L. multiflora* from Japan has also been studied, but no hexaploid has yet been reported from that part of the world. Where the tetraploid and hexaploid races were collected together in Europe and eastern North America, they were found growing mixed with each other. They were impossible to distinguish morphologically, though the tetraploid is somewhat more slender than the hexaploid. In the sample of plants collected at Bretton Woods, N. H., at the base of Mt. Washington, there were also found several hybrid plants with 30 chromosomes. That they were true hybrids was checked by studying their meiosis, in which 12 bivalents and six univalents were found at first metaphase. Plants of the more slender tetraploid race of *L. multiflora* have often been described as *L. pallescens*, with which species it has obvious similarities in many respects. An artificially made tetraploid race of *L. pallescens* is morphologically almost identical with the tetraploid *L. multiflora*, for which reason this latter species may be regarded as a polyploid of *L. pallescens*. In eastern North America we have, in addition to the two *multiflora* races, two diploid species, *L. echinata* (SMALL) HERMANN and *L. bulbosa* (WOOD) RYDBERG. These two species seem to occupy warmer places

than *L. multiflora* and their distribution reach further south. In the alpine regions of Sierra Nevada in California and probably also in the same regions in the mountains further north we have a diploid *multiflora* race, with small flowers and bright green, rather glabrous leaves. It seems to be the same species as the one described by ST. JOHN (1936) from the mountains of Oregon and Washington as *L. campestris* v. *columbiana*. Though I have not studied any specimens from the original locality, I will for the present deal with the plants from California also under this name.

Chromosome determinations of plants belonging to the *campestris-multiflora* complex have already been made in several cases. Very often, however, it is impossible to identify those species that have been used for the counts. The names *L. campestris* and *L. multiflora* have been used many times in the collective sense (SCHEERER, 1940; MALHEIROS and GARDÉ, 1947, *etc.*). The determination of *L. frigida* from different localities of Greenland made by HAGERUP (1941) and by BÖCHER and LARSEN (1950) has in both counts given the same result as that made in this investigation, *i. e.* $2n = 36$. The same is the case with *L. multiflora* determined by BÖCHER and LARSEN (1950), LÖVE and LÖVE (1944), and HAGERUP (1941). The chromosome number $2n=36$ given by DE NORONHA WAGNER (1949) for *L. pallescens* was, however, probably examined on a plant of *L. multiflora*. The material determined originated from the Botanical Gardens of Gothenburg, and the specimen of *L. pallescens* I found there last summer (1950) belonged no doubt to *L. multiflora*.

The third group investigated of the subgenus *Gymnodes* is the *arctica* group containing *L. arctica* BLYTT and the complex *L. arcuata coll.* composed of *L. arcuata* (WG.) SW. and *L. confusa* LINDEB. (see Table 3 and Fig. 4 *e—g*). *L. arctica* has $2n = 24$, all chromosomes of which seem to belong to the AL-type of *Gymnodes* (see Fig. 4 *e*). This species is accordingly to be regarded as a tetraploid of ordinary type. The complex species *L. arcuata coll.* has different chromosome patterns. The most common one is that with 36 chromosomes. The chromosomes are here of distinctly different sizes. Twelve of the chromosomes are larger than any found in the subgenus *Gymnodes*. They have the size of the AL-type chromosomes found in *Anthelaea*, *i. e.* about 2 μ long. It is impossible to decide at this stage of the investigation whether this fact indicates any relationship with that subgenus. The rest of the 36 chromosomes are smaller and seem to be of the AL- or BL-type of *Gymnodes* (see Fig. 4 *f*). This chromosome pattern of *L. arcuata* is found in plants originating from Helags in Härjedalen, Sweden, as well

as in those of Eastern Greenland and North-Eastern Canada. In the samples originating from Abisko in Lapland different chromosome patterns were found. Though the chromosome numbers were hard to determine on account of the difficulty of getting good fixations, the plants of the sample seemed to have two different chromosome patterns, one with $2n = 36$ and one with $2n = 42$. The plants with 36 chromosomes have the same chromosome pattern as the ones from Härjedalen, but in the plants with 42 chromosomes it was possible to count only six large ones (see Fig. 4 *g*). From West and South-Eastern Greenland BÖCHER and LARSEN (1950) have found plants with 36 chromosomes. They found size differences between the chromosomes in the cells of the plants, but the relation between the different types of chromosomes is not exactly the same as that found in this investigation. From North-Eastern Greenland SÖRENSEN and WESTERGAARD have investigated a plant belonging to this complex, determining the chromosome number as $2n = 48$ (LÖVE and LÖVE, 1948). In a letter last year (1950) Professor WESTERGAARD, however, informed me that on a re-examination of the chromosome number the plant was found to have only 42. The plant had a very irregular meiosis, but sometimes showed full pairing with 18 chromosome bodies at meiosis. These facts indicate that the plant investigated is of hybrid origin. Perhaps the 42-chromosomal plants from Abisko are of the same type. As they did not flower in Uppsala, no information about the behaviour of their meiosis was obtained. (For the interpretation of chromosome pairing at meiosis of *Luzula* hybrids, see below.)

PTERODES.

Pterodes, the third subgenus of the genus *Luzula*, contains relatively few species. Only eight species are known, all of which seem to be closely related. Usually they have a high chromosome number, which is sometimes aneuploid. The somatic chromosome numbers known from this subgenus are as follows: 24, 42, 48, 52, and 66 (see Table 5 and Fig. 5). *L. Forsteri* (SMITH) DC. and *L. luzulina* (VILL.) D. T. and SARNTH. [*L. flavescens* (HOST) GAUD.] have 24 chromosomes of BL-type, *i. e.* the same size as the BL-type of *Gymnodes*. All 48 chromosomes of *L. acuminata* RAF. (L. *saltuensis* FERNALD) and the majority of the 52 chromosomes of *L. rufescens* FISCHER are of CL-type. *L. Johnstonii* BUCHENAU with 42 chromosomes seems to have six of BL-type and 36 of CL-type, and *L. pilosa* (L.) WILLD. with $2n = 66$ has chromosomes that are smaller than those of CL-type. In the last-mentioned

TABLE 5. *Species, original localities, and chromosome*

Species	Localities
L. Forsteri	Normandie, France
»	Jura bei Biel, Kt. Bern, Switzerland
L. luzulina	Bot. Gardens of the Univ., Göttingen
»	Höttinger Alm, Tyrol
»	Igler Alm, »
»	Patscherkofel, »
»	» »
L. Johnstonii	Mt. Kilimanjaro, Central Africa
»	» Kenya, Central Africa
L. acuminata	Chambly Co., Que., Canada
L. rufescens	8 miles E. Dawson, Yukon, »
»	64°01′ N; 140°50′ W, Yukon, »
L. pilosa	Uppsala, Sweden
»	Between Ljungdalen and Kesudalen, Härjedalen, Sweden
»	Vådö, near Helsingfors, Finland
»	Höttinger Alm, Tyrol
»	Fischbach, Styria
»	Maria Trost bei Graz, »
»	Glashütten, Koralpe, »
»	Tübingen, Germany
»	Leeds, England

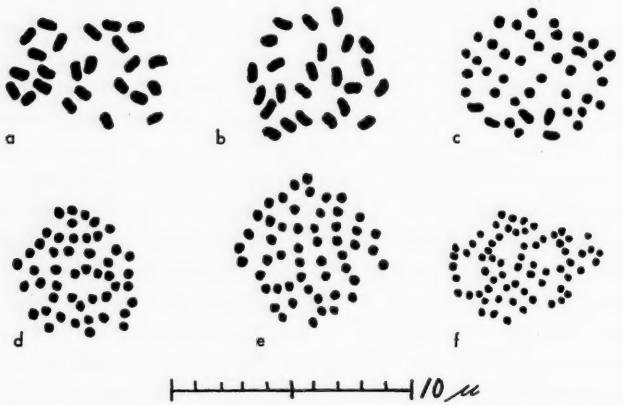

Fig. 5. Chromosome patterns of the species belonging to the subgenus *Pterodes*, shown by camera lucida drawings of their mitotic metaphases. (*a*) *L. Forsteri* $2n = 24$; (*b*) *L. luzulina* $2n = 24$; (*c*) *L. Johnstonii* $2n = 42$; (*d*) *L. acuminata* $2n = 48$; (*e*) *L. rufescens* $2n = 52$ (*f*) *L. pilosa* $2n = 66$.

numbers of the material studied in the subgenus Pterodes.

Habitat	Collector	Material collected	Numb. of chromosome determined plants	2*n*
	Rouen Bot. Gard.	seeds	3	24
	ED. BERGER	2 pl	2	24
		seeds	3	24
Open wood at 1000 m. alt.	Author	15 pl	3	24
Wood at 1300—1400 m. alt.	»	8 »	6	24
Open wood at 1700 m. alt.	»	4 »	3	24
Along brook at 1750 m. alt.	»	9 »	7	24
At 3075 m. alt.	O. HEDBERG No. 1368	seeds	2	42
In *Hypericum* copse at 3000 m. alt.	» » 1365	»	3	42
	G. DAIGNEAULT	»	7	48
	J. A. CALDER No. 4336	»	6	52
	» » 4414	»	4	52
Wood	Author	32 pl	10	66
» at abt. 700 m. alt.	»	19 »	10	66
Wood	»	11 »	9	66
Open wood	»	7 »	5	66
» »	»	4 »	3	66
Edge of wood	»	5 »	2	66
Wood	»	4 »	3	66
Beech wood	»	2 »	2	66
	H. G. BAKER	2 »	1	66

plant the chromosomes are too small to permit of a more thorough investigation. An earlier determination of the chromosome numbers of *L. Forsteri* (MALHEIROS and GARDÉ, 1947) is verified here. From a determination of the chromosome number of *L. pilosa* SCHEERER (1940) reported $2n =$ about 62; HAGERUP (1941), however, obtained $2n = 72$. The chromosome number of this species is very hard to determine on account of its very small chromosomes. However, I have never obtained such a high chromosome number as $2n = 72$ in any of the specimens I have determined from different parts of Europe. But, of course, there is a possibility that the high chromosome number of this species varies. The different species of the subgenus *Pterodes* are usually geographically separated in distribution. So far as we yet know, there are only two regions that have more than one species known from the same area. These regions are the south European mountains, where three species meet, *L. pilosa*, *L. Forsteri*, and *L. luzulina*, and Eastern Asia where at least *L. plumosa* E. MEYER and *L. rostrata* BUCHENAU meet and probably come together with *L. rufescens*.

DISCUSSION.

For the comparative study of the type and size of the chromosomes the same part of the plants, *i. e.* the root tip, has been used. Furthermore, the same fixative, chrome-acetic formalin, as well as a standardized treatment has always been applied. Despite all these precautions there is a certain degree of variation in the size of the same type of chromosomes, especially between certain sections of the genus. Then, too, between closely related species of the same chromosome pattern the difference in size of the chromosomes may be distinct. For instance, the diploids of the *campestris-multiflora* complex of West America (*L. comosa* v. *macrantha* and *L. comosa* v. *laxa*) have distinctly larger chromosomes than the diploids of that complex originating from Scandinavia (*L. campestris* and *L. pallescens;* see Fig. 3), though they easily hybridize. In the mitotic metaphase of the F_1 hybrid it is easy to distinguish six chromosomes of each size. In spite of this fact meiosis is rather regular, generally showing six bivalents. Thus, chromosomes of the same type need not always have exactly the same size, though usually their size is the most obviously distinguishing character for the chromosome classification. The pairing ability of the chromosomes at meiosis will, however, afford a conclusive test for the classification of the chromosomes. Still, on the whole, a comparison of the chromosome-size of closely related species or races will give a good survey of the different chromosome types and the relationship between them.

In summarizing the results of the investigation we will point out the evidence brought to light which most obviously shows that there are really different »chromosome types» within the genus *Luzula* and that there exists a distinct relationship between these different types of chromosomes. Perhaps the most obvious proof of that relationship is the occurrence of euploid and aneuploid chromosome races of the same species, as we have them, for instance, in *L. spicata* and *L. orestra.* Here we have a striking and simple replacement of two BL-type chromosomes in one of the chromosome races for one AL-type chromosome in another race of the same species. The series of polyploidy in which the chromosome size decreases to about half as the chromosome number rises to the double, which occurs in races of *L. spicata* and in a more complicated way in species of the *campestris-multiflora* complex and the subgenus *Pterodes*, also provides strong support for the existence of a distinct relationship between the various chromosome types.

How have these different »chromosome types» arisen in species or

races which must be regarded as closely allied? Some kind of regularly acting fusion or fragmentation of the chromosomes seems to be the most likely explanation at this stage of the investigation. If we accept the most common type of chromosome pattern with 12 chromosomes as being the original one, the fragmentation hypothesis would be the most probable one. We should then have among the species here studied a type of polyploidy in which the polyploid chromosome number is attained by some kind of fragmentation instead of by multiplication of the chromosome sets in the nucleus. As the high chromosome numbers in a polyploid series of this type seem to have been reached without any increase of the chromatin mass or chromatid length, I would propose calling this type of polyploidy an *endo-nuclear polyploidy*, in contrast to the ordinary type of polyploidy built up by added chromosome sets. The aneuploid chromosome patterns would then easily be interpreted as a half-completed endo-nuclear polyploidy. An understanding of the realization of these phenomena is facilitated by considering the facts concerning the centromere described in some *Luzula* species by CÂMARA and coworkers (MALHEIROS and DE CASTRO, 1947; MALHEIROS, DE CASTRO and CÂMARA, 1947; DE CASTRO, CÂMARA and MALHEIROS, 1949) and ÖSTERGREN (1949), and verified by other authors (BROWN, 1950; BERGER, 1950; THOMAS, 1950). In these investigations it was found that the *Luzula* species studied did not have a centromere of normal type. CÂMARA and coworkers interpreted the phenomena they observed as being due to some kind of diffuse or multipolar centromere. Such a diffuse centromere extending along the whole chromosome makes it possible for fragments to act as normal chromosomes, since they always have centromeres directing them to the poles. The *Luzula* species with this diffuse centromere was also shown to have a special type of meiosis, in which the first division is equational and the second one reductional. In the study of meiotic or mitotic divisions of the species here examined no facts have been disclosed which contradict the main statements of these workers as to the chromosome behaviour of the genus *Luzula*. On the contrary, the type of mitotic and meiotic divisions in species of different chromosome patterns as well as their hybrids support these statements. At the first anaphase of hybrids with irregular meiosis no lagging chromosomes were ever observed, even if there had been univalents during metaphase. This phenomenon is most easily explained by assuming the behaviour at meiosis for the species here examined of the genus *Luzula* to be the same as that earlier demonstrated for *Luzula* species and most thoroughly studied in *L. purpurea*. The chromosomes

of this species, which possesses diffuse centromeres, has the first meiotic division equational (MALHEIROS, DE CASTRO and CÂMARA, 1947).

A second proof of the fragmentation theory is demonstrated by the behaviour of chromosome pairing at meiosis in hybrids containing different types of chromosomes. Only two types of such hybrids have been studied up to date. The first one is between two species with 12 chromosomes of AL-type (*L. campestris* and *L. pallescens*) and one species with 48 chromosomes of CL-type (*L. sudetica*). Somatically the hybrids have six large chromosomes of AL-type and 24 small of CL-type (see Fig. 6). In the first metaphase of meiosis it is possible to observe the six large chromosomes, which never pair with each other but with several of the small ones. Some of the small chromosomes lie, however, spread around

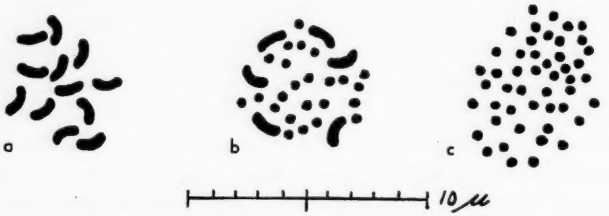

Fig. 6. Somatic metaphases of (*a*) *L. campestris* $2n = 12$; (*c*) *L. sudetica* $2n = 48$; and (*b*) the F_1 hybrid between them, $2n = 30$. Note their different chromosome patterns.

in the nucleus as univalents without any association with the large chromosomes or with each other. The second type of hybrids studied consists of those obtained between the high-chromosomal species of the subgenus *Pterodes*. The hybrid of *L. pilosa* ($2n = 66$) and *L. rufescens* ($2n = 52$) had 59 chromosomes somatically. In meiosis, however, 26 distinct chromosome bodies were observed, and only in a few cells were there 25 or 27. This fact can most easily be explained by assuming that several of the small chromosomes of *L. pilosa* become associated with the larger ones of *L. rufescens* and that the *rufescens* chromosomes are usually unable to pair with each other. The association between several small and one large chromosome would presumably be realized in the same way, as several fragments would pair with their homologous parts of a large chromosome. As a result of such a type of pairing at metaphase, the diffuse centromere of the chromosomes, and the equational first meiotic division, there is obtained that very regular first anaphase of meiosis without any lagging chromosomes which always appears in

meiosis of the hybrids. A corresponding result of chromosome pairing at first metaphase was found in the other hybrids between those species belonging to *Pterodes* which have been examined. A more thorough study of these hybrids and their meiosis will be given later. The chromosomes of the hybrids here studied are, however, unfortunately too small to allow of any detailed cytological examination.

The type of meiosis shown by the high-chromosomal species of the genus *Luzula* formed by endo-nuclear polyploidy also supports the fragmentation theory as the origin of that type of polyploidy. None of the investigated species of that kind has up to now been found to form any multivalents at first metaphase. This fact might indicate that the small chromosomes are not homologous in spite of their polyploid numbers. However, polyploids of ordinary type met with in nature have not been found to form any multivalents either. But the artificially produced tetraploid *L. pallescens*, obtained by colchicine treatment, has a few multivalents in some cells at first metaphase of meiosis.

The type of chromosome patterns described for various species of the genus *Luzula* is also likely to be found in other related genera, *e. g.* in *Juncus*, *Carex*, *Scirpus*, *etc.*, as can be educed from descriptions of the chromosomes of these genera in the available literature (WULFF, 1939; HEILBORN, 1939; WAHL, 1940; HÅKANSSON, 1928, 1929; TANAKA, 1938, 1939, 1949, *etc.*). This relationship between chromosomes described in various chromosome patterns found in the genus *Luzula* would thus give a likely explanation of the well-known phenomenon of aneuploid chromosome numbers in these genera, especially *Carex* and *Scirpus*. The chromosome patterns of the three races of *Scirpus lacustris* described by TANAKA (1938) show the same relationship between chromosome numbers and chromosome sizes as is found in the aneuploid chromosome races described in various *Luzula* species. In the genus *Carex* the high-chromosomal species have on an average smaller chromosomes than the low-chromosomal ones (HEILBORN, 1939), which is the case in the genus *Luzula*. The same type of meiosis as the one described in *Luzula* seems to be found in *Carex* as well. In his description of the meiosis of *Carex* hybrids WAHL (1940) especially points out that lagging chromosomes were never found at first anaphase in spite of the fact that the chromosome pairing at first metaphase had been irregular with multivalents and univalents. He also interprets this phenomenon as being due to the fact that the first meiotic division must be equational.

Acknowledgements. — The author wants to express her gratitude to all colleagues and friends who have helped to bring together the

material for this study and made it possible to carry out this investigation by sending seeds and living plants from different parts of the world.

SUMMARY.

(1) The chromosome numbers of the following species of the genus *Luzula* have been determined and the results are given below (compare Tables 1—5 for origin and localities).

Subgenus Anthelaea:

L. purpurea LINK. $2n = 6$
L. silvatica (HUDS.) GAUD. $2n = 12$
L. lutea (ALL.) DC. $2n = 12$
L. Henriquezii DEG. $2n = 12$
L. luzuloides (LAM.) DANDY and WILM. $2n = 12$
L. nivea LAM. and DC. $2n = 12$
L. parviflora (EHRH.) DESV. $2n = 24$
L. glabrata DESV. $2n = 12$
L. Wahlenbergii RUPR. $2n = 24$
L. spadicea (ALL.) DC. $2n = 12$
L. subcongesta JEPS. $2n = 24$

Subgenus Pterodes:

L. Forsteri (SMITH) DC. $2n = 24$
L. luzulina (VILL.) D. T. and SARNTH. $2n = 24$
L. Johnstonii BUCH. $2n = 42$
L. acuminata RAF. $2n = 48$
L. rufescens FISCH. $2n = 52$
L. pilosa (L.) WILLD. $2n = 66$

Subgenus Gymnodes:

L. spicata (L.) DC. $2n = 12, 14, 24$
L. abyssinica PARL. $2n = 24$
L. arctica BLYTT. $2n = 24$
L. arcuata (WG.) SW. *coll.* $2n = 36, 42$

Campestris-multiflora complex:

L. campestris (L.) DC. *s. str.* $2n = 12$
L. pallescens SW. $2n = 12$
L. echinata (SMALL) HERM. $2n = 12$
L. bulbosa (WOOD) RYDB. $2n = 12$
L. comosa v. *macrantha* WATS. $2n = 12$
L. comosa v. *laxa* BUCH. $2n = 12$
L. campestris v. *columbiana* ST. JOHN. $2n = 12$
L. sudetica (WILLD.) DC. $2n = 48$
L. orestra SHARSM. *ined.* $2n = 20, 22$
L. groenlandica BÖCHER. $2n = 24$
L. campestris v. *vallesiaca* BEAUV. $2n = 36$
L. comosa E. MEY. *s. str.* $2n = 24$
L. multiflora (RETZ.) LEJ. $2n = 24, 36$
L. frigida s. SAM. $2n = 36$
L. congesta (THUILL.) LEJ. $2n = 36, 48$

(2) About one-third of the species investigated have 12 chromosomes of equal size in the nucleus. The chromosome pattern of this kind has been called the standard chromosome pattern and its chromosomes are designated the AL-type of chromosomes of the genus *Luzula*.

(3) Three different kinds of chromosomal deviation from the standard pattern have been traced:

(a) An ordinary type of polyploidy in which the standard chromosome sets are doubled twice, three or four times to tetraploids, hexaploids or octoploids. Chromosomes of AL-type are found throughout in all these polyploids.

(b) A special type of polyploidy in which the chromosome size regularly decreases when the number of chromosomes increases has been described. When the chromosome number is doubled from 12 to 24, the size of the chromosomes is reduced to about half that of the AL-type. Chromosomes of this reduced size are called the BL-type. When the chromosome number is doubled from 24 to 48 the chromosome size is again reduced to about half that of the BL-type. These small chromosomes are called the CL-type. Further reduction of the chromosome size is met with in connection with increased chromosome number. This special type of polyploidy has been called *endo-nuclear polyploidy*. The two types of polyploidy here described may sometimes form a compound polyploidy, as, for instance, in *L. campestris* v. *vallesiaca* with $2n = 36$, containing 12 chromosomes of AL-type and 24 of BL-type.

(c) Chromosome races of certain species show a particular type of aneuploidy, which has been interpreted as a half completed endo-nuclear polyploidy. In the chromosome patterns of these aneuploids two chromosomes of BL-type replace one of AL-type in the standard chromosome pattern, and two of CL-type correspond to one of BL-type in the endo-nuclear polyploids.

(4) The endo-nuclear polyploidy or its related type of aneuploidy is found in all three subgenera of the genus *Luzula*; ordinary polyploidy has up to date only been found in the subgenus *Gymnodes*.

(5) As a plausible explanation of these chromosomal deviations from the standard type a regularly acting fragmentation seems to be the most likely. The diffuse centromere shown by the genus *Luzula* will ensure the survival of the fragmented chromosomes in the cells.

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CYTOGENETICAL STUDIES ON TRANSLOCATIONS IN PISUM

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INTRODUCTION.

THE genetical analysis of structural hybrids may be useful in connection with the mapping of chromosomes. In *Pisum sativum* a number of spontaneous interchange types are known, and as four of these have been studied both cytologically and genetically they are especially suitable for the purpose mentioned above. Using the designations given by NILSSON (1936), the following types are concerned in the present investigations:

Prime type A = the normal type = structural type 1 (SANSOME, 1937)
» » B = HAMMARLUND's K-line = structural type 2 (SANSOME, 1937)
» » C = Thibet 7 = structural type 3 (SANSOME, 1937)
» » D = Extra Rapid
» » E = x-normal from N III

It should be pointed out that Extra Rapid has been called structural type 4 by SANSOME (1937). Prime type D and structural type 4 do not, however, belong to the same structural type (LAMM, 1949; SANSOME, 1950). A series of meiotic investigations made by HÅKANSSON (1929, 1931, 1932, 1934), SANSOME (1932, 1933, 1937, 1938 a), and PELLEW and SANSOME (1931) on hybrids between the prime types A—E has also been completed. In this paper I am suggesting that prime type E may be identical with structural type 4.

Miss C. PELLEW most kindly has supplied me with new genetical data concerning the prime type C. These data are most valuable for the interpretation of the cytological investigations, and I am grateful for the permission to publish them in the genetical part of this paper. I also wish to express my indebtness to Professor F. NILSSON for providing me with facility to carry out the *Pisum* investigations at the Horticultural Research Station at Alnarp, and I would like to thank Mr. R. HOCHBERGS for technical assistance.

CYTOLOGICAL STUDIES.

METHODS.

Crosses between some of the prime types have been made during 1949 and 1950. The F_1 plants have been grown in green-houses usually during the winter. Flower buds were collected for cytological studies of meiosis and smears were made of the anthers. The technique employed is the same as that used by LIMA-DE-FARIA (1948) on anthers of rye. He has kindly given me instruction and help. The material was fixed in acetic alcohol 1 : 4 for four hours, afterwards treated for about twelve hours with 95 % alcohol and then stored in 70 % alcohol. The smears were stained with aceto-carmine. As already noted by HÅKANSSON (1931, p. 42), the contraction of the chromosomes may be quite variable. A few buds were fixed for only two hours and afterwards immediately stained. This treatment resulted in less swollen chromosomes. The technique employed gave poor preparation of pachytene but good results for the later meiotic stages. Perhaps the nigrosine method described by v. ROSEN (1949) would have been better for pachytene.

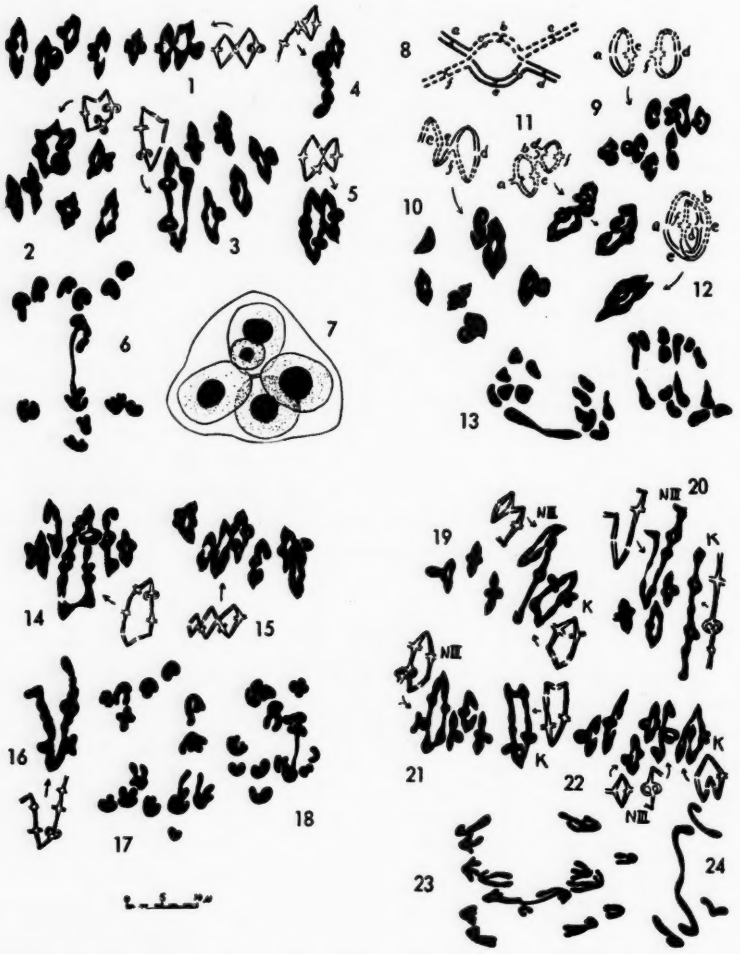
The fertility of pollen from mature anthers has been studied in aceto-carmine glycerine 1 : 1.

HYBRIDS STUDIED.

Prime type D × A. — In the summer of 1949 I made crosses between prime type D (line 83) and the normal structural type (lines 3 and 26). It should be mentioned that Dr. E. NILSSON has supplied me (and had also earlier supplied SANSOME) with seed of the prime types D and E. Originally NILSSON bought seed of Extra Rapid (prime type D) from the Seed Company W. WEIBULL, Landskrona, Sweden. This variety was released in 1927 from the Plant Breeding Institute of Weibullsholm. As to the origin of prime type E, see NILSSON (1936).

The semisterility of the cross D × A was originally detected by HAMMARLUND, and cytologically investigated by HÅKANSSON (1931). HÅKANSSON found five bivalents and one association of four chromosomes. Rings of four were more common than chains, and of the former about equal numbers showed adjacent and alternate disjunction. Numerical non-disjunction seems to occur frequently (HÅKANSSON, 1936 b) and this gives viable female gametes with eight (n + 1) chromosomes. In addition to these earlier descriptions I have made some new observations to complete the picture.

Figs. 1—3 show three complete nuclei at M I of the F_1 hybrid be-



Figs. 1—24. Meiosis in the PMC's of some translocation heterozygotes. — Figs. 1—7, from prime type D × A. — Figs. 1—3, complete nuclei with one ring of four chromosomes and five bivalents. — Figs. 4—5, configurations of four with chiasmata in an interstitial segment. — Fig. 6, A I with inversion bridge and fragment. — Fig. 7, tetrads with one micronucleus (half magnifications as compared with the other figures). — Figs. 8—13, from prime type D × C. — Fig. 8, probable pachytene diagram of the configuration of four (chiasmata indicated by *a—f*). — Figs. 9—10, complete nuclei, the former with seven bivalents, the latter with five bivalents and one configuration of four. — Figs. 11—12, configurations of four. — Fig. 13, A II with sticky chromosomes. — Figs. 14—18, from prime type D × B. — Figs. 14—15,

tween the lines 83 and 26. The rings of four are typical. One of the chromosomes of the ring seems to have two rather long arms, both sometimes taking part in more than one chiasma (Fig. 2). A chiasma may now and then be formed in the interstitial segment between the centromere and the point of interchange (*cf.* LAMM, 1948) in one pair of chromosomes of the association (Figs. 4—5). A similar behaviour has been observed in the translocation heterozygote between prime types A and C (SANSOME, 1933, p. 21), whereby the ring of four takes the form of a figure-of-eight. At A I and A II an inversion bridge with a big fragment has been observed in a few PMC's; this is shown in Fig. 6. At the tetrad stage micronuclei often occur. About 10 % of the tetrads contained one micronucleus (Fig. 7). Tetrads with two micronuclei were more rare.

In the translocation heterozygote pollen abortion was 51 % (n = 1382) and ovule abortion was 57 % (n = 797).

Prime type D × C. — From Dr. E. NILSSON I obtained seed of Thibet 7 and in 1949 I crossed this line with Extra Rapid (line 83). Meiosis of the F_1 hybrid was characterized by five bivalents and one peculiar configuration of four chromosomes (Fig. 10). The abortion of pollen and ovule were both found to be 65 % as determined on 1311 pollen grains and 733 ovules. A ring of four chromosomes was never observed. The existing configurations could be interpreted by assuming the occurrence of two interchanges involving two chromosomes. The probable pachytene configuration is illustrated by Fig. 8. If no chiasmata are formed in the differential segments (*i. e.* chiasmata *b* and *e* of Fig. 8 are failing) only bivalents are formed (Fig. 9). This is not too rare. Figs. 10—11 demonstrate common types of configurations at M I, whereas the type shown in Fig. 12 is more rare. At A I there are no inversion bridges. Bridges seen at A II (Fig. 13) are probably due to stickiness. Bridges of a similar type have been observed in an asynaptic strain of *Pisum sativum* (*cf.* KOLLER, 1938, p. 291, Fig. 42).

According to SANSOME (1937 and 1938 a) structural type 3 (= Thibet 7) and 4 (= Extra Rapid) should have only one chromosome in common, and hence a ring of six should be expected. The disagreement between her and my own results can be explained by the assumption

complete nuclei with four bivalents and one ring of six. — Fig. 16, chain of six. — Figs. 17—18, A I without and with inversion bridge. — Figs. 19—24, from prime type E × B. — Figs. 19—21, complete nuclei with three bivalents and two associations of four (N III and K). — Fig. 22, the N III association split into one open and one ring bivalent. — Fig. 23, A I with inversion bridge and fragment. — Fig. 24, the inversion bridge and the fragment at A II.

that there are two different structural types in the Extra Rapid variety (SANSOME, 1950).

Prime type D × B. — The cross between Extra Rapid (line 83) and HAMMARLUND's K-line was made in 1950. F_1 plants were raised the same year. At M I the PMC's contained four bivalents and one configuration of six chromosomes (Figs. 14—15). This hybrid is thus characterized by two interchanges involving three chromosomes. That the two prime types have one chromosome in common was assumed by LAMM (1948). It is possible to recognize in the configurations of six, illustrated by Figs. 14—16, the Extra Rapid chromosome with two long arms and the small K-line chromosome with short arms (see HÅKANSSON, 1931 and SUTTON, 1939). The differential segment seems to be too short for chiasma formation. In this respect the ring of six is different from the corresponding well known ring of prime type C × B described by SANSOME (1932 and 1933).

A I was usually normal (Fig. 17) but sometimes an inversion bridge with a fragment was observed (Fig. 18). It is rather similar to the bridge and fragment found in F_1 between prime type D and A (Fig. 6). The gametic abortion of pollen (n = 706) and of ovules (n = 1556) were both 68 %.

Prime type E × B. — F_1 of this cross has been made and studied by me in 1950. Seed of the two prime types was obtained from Dr. E. NILSSON. By kind permission of Professor H. BURSTRÖM I was allowed to raise the F_1 plants in a green-house at the Botanical Institute of the University of Lund. The temperature was comparatively low and the plants obtained additional electrical light. They flowered in December and gave remarkably good fixations.

In the PMC's of this cross two associations of four were frequently found (Figs. 19—22). Sometimes both of them consisted of rings (Fig. 21), sometimes of chains (Fig. 20). One ring and one frying-pan-like configuration with a triple chiasma were very common (Fig. 19). From the descriptions of the amphibivalents of the crossings prime type B × A and E × A (= the N III case) given by HÅKANSSON (1929, 1931 and 1932) it was easy to classify these configurations. This has been done in Figs. 19—22 where they are marked with K and N III. The former are comparatively regular, and although the size of chromosome segments is difficult to determine in meiotic metaphase chromosomes the rings evidently have one chromosome with short arms and one with long arms in opposite positions (Figs. 19, 21 and 22).

The N III configurations, which according to HÅKANSSON (1932)

most frequently occur in translocation heterozygotes between prime types E and A, are of the type which according to MARQUARDT (1948) would be expected if chiasmata are formed in one of the interstitial segments and often fail in some of the distal segments. Presumably the segmental interchange involves a short distal segment of a long chromosome arm and the greater part of a chromosome arm of intermediate length. In the translocation heterozygote chiasmata often fail in the former segment and sometimes also in the latter, whereby the configuration is split into two bivalents (Fig. 22), one of which is of the rod type (*cf.* HÅKANSSON, 1932). The same and similar but more complicated types of configurations have been found in some *Pisum* hybrids by SANSOME (1938 b).

The frying-pan-configuration so often seen at meiosis in E × B hybrids seems also to occur in the hybrids between SANSOME's line of Extra Rapid (structural type 4) and HAMMARLUND's K-line (*cf.* SANSOME, 1950, Fig. 1 *b*). If she has not exchanged Extra Rapid and prime type E, both of which were originally obtained from Sweden, there must be two different structural prime types within the variety Extra Rapid.

At A I and A II an inversion bridge with a large fragment was sometimes observed (Figs. 23—24). The gametic abortion of the hybrid between prime type E and B has only been determined from pollen grains (n = 1028) and was 70 %.

Additional cases. — In addition to the investigation described above it should be mentioned that, according to a letter from Professor A. HÅKANSSON to Dr. E. NILSSON written in Lund on March 23, 1940, there is an association of six chromosomes in the hybrid between prime type E and C. I have kindly been permitted to publish this observation. There is also an association of six chromosomes in the crossing of prime type E × D (HÅKANSSON, 1934), generally occurring as a chain but sometimes as a ring of six.

SUMMARY OF CYTOLOGICAL INVESTIGATIONS.

A survey of the meiotic investigations is given below. The combinations between prime type A and any of the other prime types (B—E) have been excluded, these are all of a more or less simple type with five bivalents and one configuration (often a ring) of four chromosomes and with about 50 % gametic abortion. In accordance with SANSOME's list of segmental interchange lines in *Pisum sativum* (SANSOME, 1937 and 1938 a) her numbers denoting the structural types in question and the chromosomes involved are also given in the scheme. The approximate

gametic abortion of the different structural heterozygotes is also mentioned. Structural type 4 is registered as Extra Rapid S., and prime type D as Extra Rapid N.

As to the prime type E, chromosome 3 is certainly involved, and this will be discussed later. If Extra Rapid S. and prime type E are identical the other chromosome involved should be chromosome 5. By extending the analysis to some other prime types (*i. e.* F and G) it will be possible to test the assumption concerning chromosome 5.

Survey of the results of the meiotic investigations.

Prime types	B	C	D
Structural types	2	3	
Names	K-line	Thibet 7	Extra Rapid N.
Chromosomes	1 and 2	1 and 3	1 and 3
(E?) 4 Extra Rapid S. 3 and 5	3 II + 2 IV (SANSOME, 1950)	4 II + 1 VI (SANSOME, 1950)	Not studied
E (4?) from N III 3 and (5?)	3 II + 2 IV (LAMM, 1951) 70 %	4 II + 1 VI (HÅKANSSON, unpubl.)	4 II + 1 VI (HÅKANSSON, 1934) > 50 %
D — Extra Rapid N. 1 and 3	4 II + 1 VI (LAMM, 1951) 68 %	5 II + 1 IV (LAMM, 1951) 65 %	(peculiar type)
C 3 Thibet 7 1 and 3	4 II + 1 VI (SANSOME, 1932 and 1933) 70 %		

A correct interpretation of the inversion bridge occurring in some of the translocation heterozygotes could only be obtained by further studies. As a working hypothesis it might be assumed that one of the chromosomes involved contains a duplication which in structurally homozygous plants does not or very rarely interfere with the normal pairing. HÅKANSSON (1931, p. 36) has sometimes observed bridges in plants with normal pairing. In the translocation heterozygote, however, the restricted normal pairing in the neighbourhood of the point of interchange may increase the chances for differential pairing, whereby the free chromosome arm is perhaps folded back in a position making the pairing between the duplicated segments possible.

In hybrids between the prime types A and B, bridges were not found by HÅKANSSON (1931). Hence, chromosome 1 and 2 are probably not involved. I have observed inversion bridges in some of the cases where chromosome 3 is involved in

the interchanges (*i. e.* D × A, D × B and E × B). Perhaps there is a duplication in this chromosome. This chromosome is probably also involved in some of the structural types of *Pisum humile* (SANSOME, 1938 b, p. 497). Inversion bridges in *Pisum humile* × *sativum* crosses have been found by HÅKANSSON (1936 a) and SUTTON (1937).

GENETICAL STUDIES ON PRIME TYPE C (THIBET 7).

Genetical and cytological studies on the Thibet 7 translocation in *Pisum* have previously been published by PELLEW and SANSOME (1931). The genetical part of these studies has been continued by Miss PELLEW at the John Innes Horticultural Institution, London, and later in 1942—1943 at the Rockefeller Field Station, Cambridge, by kind permission of Professor F. T. BROOKS. A survey of these crosses made by Miss PELLEW is given below and the results of the crosses are summarized in Table 1. These results have in turn been utilized for the combined estimations of the linkage relations given below the table.

Survey of crosses made by Miss C. PELLEW *for studies of the Thibet 7 translocation.*

Nos. of crosses as registered by LAMM	PELLEW	Prime types (A = normal, C = Thibet 7) and genes involved
BC 1	131/33	$\frac{\text{(A) } a\ i\ r\ st}{\text{(A) } a\ i\ r\ st} \times \frac{\text{(A) } a\ i\ r\ st}{\text{(C) } A\ I\ R\ St}$
» 2	180/33	$\frac{\text{(A) } a\ i\ r}{\text{(A) } a\ i\ r} \times \frac{\text{(A) } a\ i\ r}{\text{(C) } A\ I\ R}$
» 3	61/43	$\frac{\text{(A) } a\ B\ le\ St}{\text{(C) } A\ b\ Le\ st} \times \frac{\text{(A) } a\ b\ le\ st}{\text{(A) } a\ b\ le\ st}$
» 4	63/43	$\frac{\text{(A) } a\ B\ le\ St}{\text{(C) } A\ b\ Le\ st} \times \frac{\text{(C) } A\ b\ le\ st}{\text{(C) } A\ b\ le\ st}$
F_2 1	60, 42, 46/42	$\frac{\text{(A) } a\ B\ le\ r\ St}{\text{(C) } A\ b\ Le\ R\ st}$ selfed
» 2	356, 362/40	$\frac{\text{(A) } bt\ le\ r}{\text{(C) } Bt\ Le\ R}$ »
» 3	615/26 [1]	$\frac{\text{(A) } r}{\text{(C) } R}$ »
» 4 a	25, 29, 33, 37, 53/42	$\frac{\text{(C) } Bt\ Le\ R}{\text{(C) } bt\ le\ r}$ »
» 4 b	37/42	$\frac{\text{(C) } b\ Le\ R\ st}{\text{(C) } B\ le\ r\ St}$ »

Nos. of crosses as registered by LAMM	PELLEW	Prime types (A = normal, C = Thibet 7) and genes involved	
F_2 5	57/42	(C) *b Bt Le st* / (C) *B bt le St*	selfed
» 6	348/39	(C) *B r St* / (C) *b R st*	»
» 7	339, 341/40	(C) *B r St* / (C) *b R st*	»
F_3 1	230, 233/28 [1]	(A) *r* / (C) *R*	»

[1] From PELLEW and SANSOME, 1931.

TABLE 1. *Segregation. I, in the translocation heterozygote between prime type A and C (P. i. = point of interchange, F = fertile, S = semisterile); II, within the prime type C.*

Crossing	P. i. A AB	Phase	F *A* *AB*	F *a* *Ab*	S *A* *aB*	S *a* *ab*	n n	χ^2F χ^2A	χ^2A χ^2B	χ^2L χ^2L
1	2	3	4	5	6	7	8	9	10	11
I										
BC 1	*P. i. A*		24	19	27	22	92	0,391	1,087	0,000
»	*P. i. I*		20	23	31	18	92	0,391	1,087	2,783
»	*P. i. R*		0	43	49	0	92	0,391	0,391	92,000***
»	*P. i. St*		11	32	34	15	92	0,391	0,043	17,391***
»	*AI*	C	26	25	25	16	92	1,087	1,087	0,696
»	*AR*	C	27	24	22	19	92	1,087	0,391	0,000
»	*ASt*	C	23	28	22	19	92	1,087	0,043	0,696
»	*IR*	C	31	20	18	23	92	1,087	0,391	2,783
»	*ISt*	C	30	21	15	26	92	1,087	0,043	4,348*
»	*RSt*	C	34	15	11	32	92	0,391	0,043	17,391***
BC 2	*P. i. A*		28	22	27	26	103	0,087	0,476	0,243
»	*P. i. I*		23	27	33	20	103	0,087	0,786	2,806
»	*P. i. R*		1	49	53	0	103	0,087	0,243	99,039***
»	*AI*	C	27	28	29	19	103	0,476	0,786	1,175
»	*AR*	C	27	28	27	21	103	0,476	0,243	0,476
»	*IR*	C	34	22	20	27	103	0,786	0,243	3,505
BC 3	*P. i. A*		12	9	14	10	45	0,200	1,089	0,022
»	*P. i. B*		12	0	0	14	26	0,154	0,154	26,000***
»	*P. i. Le*		9	12	9	15	45	0,200	1,800	0,200
»	*P. i. St*		17	4	9	15	45	0,200	1,089	8,022**
»	*ALe*	C	10	16	8	11	45	1,089	1,800	0,200
»	*ASt*	R	14	12	12	7	45	1,089	1,089	0,200
»	*BLe*	R	5	7	5	9	26	0,154	1,385	0,154
»	*BSt*	C	11	1	3	11	26	0,154	0,154	12,462***
»	*LeSt*	R	12	6	14	13	45	1,800	1,089	0,556

Crossing	P. i. A AB	Phase	F*A* *AB*	F*a* *Ab*	S*A* *aB*	S*a* *ab*	n n	χ^2F χ^2*A*	χ^2*A* χ^2*B*	χ^2L χ^2L
1	2	3	4	5	6	7	8	9	10	11
BC 4	*P. i. B*		0	8	11	0	19	0,474	0,474	19,000***
»	*P. i. Le*		5	3	4	7	19	0,474	0,053	1,316
»	*P. i. St*		2	6	8	3	19	0,474	0,053	4,263*
»	*BLe*	R	4	7	5	3	19	0,474	0,053	1,316
»	*BSt*	C	8	3	2	6	19	0,474	0,053	4,263*
»	*LeSt*	R	4	5	6	4	19	0,053	0,053	0,474
F_2 1	*P. i. A*		41	12	44	10	107	0,009	1,125	0,252
»	*P. i. B*		18	23	43	1	85	0,106	0,475	32,475***
»	*P. i. Le*		42	11	42	12	107	0,009	0,701	0,028
»	*P. i. R*		33	20	51	3	107	0,009	0,900	4,265*
»	(*Rr*)		6		51			0,009	0,757	75,899***
»	*P. i. St*		32	21	43	11	107	0,009	1,374	5,237*
»	*ALe*	C	64	21	20	2	107	1,125	0,701	1,746
»	*AR*	C	66	19	18	4	107	1,125	0,701	0,084
»	*ASt*	R	57	28	18	4	107	1,125	1,374	2,103
»	*BLe*	R	42	19	22	2	85	0,475	0,004	5,188*
»	*BR*	R	42	19	24	0	85	0,475	0,318	9,894**
»	*BSt*	C	47	14	10	14	85	0,475	2,859	13,334***
»	*LeR*	C	71	13	13	10	107	0,701	0,701	7,154**
»	*LeSt*	R	56	28	19	4	107	0,701	1,374	2,493
»	*RSt*	R	56	28	19	4	107	0,701	1,374	2,493
F_2 2	*P. i. Bt*		36	15	43	19	113	1,071	1,561	0,073
»	*P. i. Le*		38	13	54	8	113	1,071	2,481	2,835
»	*P. i. R*		28	23	56	6	113	1,071	0,027	18,410***
»	*BtLe*	C	65	14	27	7	113	1,561	2,481	0,025
»	*BtR*	C	61	18	23	11	113	1,561	0,027	1,346
»	*LeR*	C	76	16	8	13	113	2,481	0,027	14,396***
F_2 3	*P. i. R*		25	16	31	3	75	0,653	0,004	9,000**
»	(*Rr*)		9		28			0,653	0,013	27,001***
F_3 1	*P. i. R*		76	43	122	6	247	0,328	3,510	33,265***
»	(*Rr*)		(16)		(116)			0,328	3,511	150,036***
II										
F_2 4 a	*BtLe*	C	153	53	45	11	262	1,837	0,046	0,748
»	*BtR*	C	167	39	39	17	262	1,837	1,837	3,137
»	(*Rr*)	C	106		31			1,837	1,840	6,293*
»	*LeR*	C	157	41	49	15	262	0,046	1,837	0,205
»	(*Rr*)	C	109		28			0,046	1,840	2,770
F_2 4 b	*BLe*	R	24	6	8	6	44	1,091	0,121	3,273
»	*BSt*	C	26	4	4	10	44	1,091	1,091	21,374***
»	*LeSt*	R	23	9	7	5	44	0,121	1,091	1,010

Cross-ing	P. i. A AB	Phase	FA AB	Fa Ab	SA aB	Sa ab	n n	χ^2F χ^2A	χ^2A χ^2B	χ^2L χ^2L
1	2	3	4	5	6	7	8	9	10	11
F_2 5	*BBt*	R	28	13	9	2	52	0,410	0,410	0,855
»	*BLe*	R	31	10	9	2	52	0,410	0,103	0,137
»	*BSt*	C	33	8	5	6	52	0,410	0,103	6,002*
»	*BtSt*	R	27	10	11	4	52	0,410	0,103	0,000
»	*LeSt*	R	29	11	9	3	52	0,103	0,103	0,034
F_2 6	*BR*	R	26	13	14	1	54	0,222	0,025	4,354*
»	*BSt*	C	34	5	5	10	54	0,222	0,222	18,181***
»	*StR*	R	28	11	12	3	54	0,222	0,025	0,403
F_2 7	*BR*	R	58	25	26	0	109	0,076	0,248	9,200**
»	*BSt*	C	70	13	11	15	109	0,076	0,027	18,032***
»	*StR*	R	58	23	26	2	109	0,027	0,248	5,138*

Combined estimations of linkage by the maximum likelihood method from the data of Table 1 are given below:

I, in the translocation heterozygote	Combined estimates from	Per cent of crossing-over
P. i. B	BC 3—4, F_2 1	1,0 ± 1,06
» *I*	BC 1—2	41,5 ± 3,53
» *R*	BC 1—2, F_2 1—3, F_3 1	5,5 ± 0,75
» *St*	BC 1, 3—4, F_2 1	27,0 ± 3,30
B R	F_2 1	Absolute
B St	BC 3—4, F_2 1	25,9 ± 4,22
I R	BC 1—2	41,0 ± 3,52
I St	BC 1	39,1 ± 5,09
Le R	F_2 1—2	28,2 ± 3,70
R St	BC 1, F_2 1	30,7 ± 4,18
II, within prime type C		
B R	F_2 6—7	16,1 ± 7,61
B St	F_2 4 b, 5—7	23,3 ± 3,05
Bt R	F_2 4 a	39,5 ± 3,65
St R	F_2 6—7	34,8 ± 6,77

In Table 1 the tests for linkage between the point of interchange and the allelomorphs for round (*R*)—wrinkeled (*r*) cotyledons have been completed by some F_2 and F_3 data already published by PELLEW and SANSOME (1931, Tables I and II, excepting F_3 families with less than 90 % germination). For the detection and estimation of linkage the methods described in my recent paper about calculations from semi-sterility data (LAMM, 1950) have been followed. In that paper empirical Ip values have been used for the testing of heterogeneity of the combined linkage data. Alternatively, however, theoretical Ip values could

be used (*cf.* BHAT, 1948). In combined estimations involving families showing absolute linkage (*i. e.*, *P. i. B* from BC 3—4 and F_2 1) I have used the theoretical Ip values. The strength of linkage has been determined only in cases giving a significant χ^2 for linkage.

Obviously the chromosomes carrying the genes *B*, *St* and *R* are involved in the Thibet 7 translocation. In one of these chromosomes, *i. e.* chromosome 3, the point of interchange is situated slightly to the left of the *B*—*St* segment. In the other chromosome (probably chromosome 1) this point is situated in the neighbourhood of the *R* locus (actually between *Pa* and *R*, according to unpublished data from investigations made by Dr. E. NILSSON). Within the prime type C the linkage data give the following order of genes: *Bt*—39,5 %—*R*—16,1 %—*B*—23,3 %—*St*. It should be noted that *Bt* and *R* in prime type A, according to NILSSON (1939), give about 37 % of crossing-over.

The other linkage relations are more doubtful and at present difficult to explain. In the translocation heterozygote there is thus a linkage between *Le* and *R*. Since there is no linkage between *Bt* and *Le* (see Table 1), *Le* cannot be situated between *Bt* and *R*. If, on the other hand, *Le* should be situated on one of the other arms of the interchange configuration it is curious that there is no significant linkage relation between *Le* and the point of interchange. The linkage relations between this point and *I* as well as the *I*—*R* and *I*—*St* relations are also astonishing. Both with respect to *Le* and *I* it is desirable to increase the strength of the inductive support now available by means of further investigations. The assumed linkage between the point of interchange and the gene *A* (PELLEW, 1937) is not supported by the data of Table 1.

CYTOGENETICAL DISCUSSION.

If the association of four chromosomes in the hybrid between prime types C and D has been correctly interpreted, chromosome 1 and 3 (according to the numbers proposed by SANSOME, 1937) are involved in both of these interchanges. This chromosome contains the *B*—*St* segment and is called chromosome III or the *B*-chromosome by LAMPRECHT (1948). According to the results of the present and earlier studies (LAMM, 1948 and 1949; NILSSON, 1950), the order of the points of interchanges and genes should be: *P. i.* (D)—*St*—*P. i.* (E)—*B*—*P. i.* (C).

Several lines of evidence indicate that the centromere is situated in the *St*—*B* segment, probably between *St* and *P. i.* (E). This would explain the reduction of crossing-over in the *St*—*B* segment of the prime

type D × A translocation heterozygote as compared with the more normal values in the C × A and E × A heterozygotes.

In the *B*-chromosome the variation of linkage, according to LAMPRECHT (1948), is high in the *St—B* segment and especially high in the *B—Gl* segment, which according to his hypothesis concerning the variation of linkage and the course of crossing-over, should indicate that *Gl* is situated near the end of the chromosome. Against this hypothesis it might, however, be said that in general the centromere region is the most variable in crossing-over properties (see MATHER, 1938 and 1939) and it is not very probable that *Pisum* is an exception to this rule.

Dr. E. NILSSON (unpublished) has found rather different ratios in the segregation of *St* and *B* in trisomics derived from the A × D translocation heterozygote, which strongly support the assumption that the centromere is situated between *St* and *B*. In this connection it should be mentioned that suggestions given by SUTTON (1939, p. 475) as to the structure of this particular trisomic type are probably wrong. Cytogenetical data concerning the trisomic studies made by NILSSON will be published later in a special paper.

Finally, the configurations found in the prime type B × C and B × D hybrids agree with the assumed localization of the centromere in the *St—B* segment and also indicate that the two arms of chromosome 3 are both comparatively long.

Chromosome 1 of prime type A by PELLEW (1937) was supposed to carry the gene *A*. This chromosome is involved in the B and D and probably also in the C interchange. Whereas *P. i.* (B) and *P. i.* (D) are both closely coupled to the gene *Gp*, *P. i.* (C) is closely coupled to the gene *R*. Further, there is a strong linkage between *P. i.* (B) and the gene *A*. Since chromosome 1 and 2 are involved in the prime type B interchange (SANSOME, 1937), the gene *A* must be carried by chromosome 2, which probably has one short and one long arm. Chromosome 1 contains the segments *Gp—P. i.* (D)—*Fs* (LAMM, 1949) and probably also *Pa—P. i.* (C)—*R—Bt*. According to LAMPRECHT (1948), the former segment is ascribed to chromosome V (the *Gp*-chromosome) and the latter to chromosome VII (the *R*-chromosome), where he gives the order *R—Pa—Bt* which, however, according to NILSSON (1950) should be *Pa—R—Bt*. Future studies of the linkage relations between *P. i.* (C) and the genes of the *Gp—Fs* segment will probably reveal if *R* is carried by chromosome 1.

The configurations observed at meiosis in the translocation heterozygotes studied involving chromosome 1 suggest that this chromosome has one short and one long arm. Probably the *Fs—Ast* segment belongs

to the former arm, and the *R—Bt* segment to the latter arm, whereas the centromere would be situated in the neighbourhood of the gene *Gp*. A supporting piece of evidence is the high variation of linkage found by LAMPRECHT (1948) in the *Cp—Gp* segment. Moreover, data published by LAMPRECHT (1951 b) reveal a negative interference between the *Cp—Gp* and the *Gp—Cr* segments. In conformity to conditions found in *Drosophila* (quoted by LUDWIG, 1938, pp. 106—111) this unusual type of interference indicates the position of the centromere within one of these segments. In this connection it should be remembered that the chromosomes of *Pisum* form nucleoli at their centromeric regions (HÅKANSSON and LEVAN, 1942). These regions are perhaps heterochromatic, which would explain the variable frequency in crossing-over (*cf.* MATHER, 1939).

Finally a few words should be said about the prime type E. Besides the linkage between *P. i.* (E) and *B—St*, NILSSON (1950) also found linkage between the point of interchange and the segments *V—Le*, *N—Fa* and *Wlo—P*. In chromosome IV the order of the genes should be *N—Fa—Le—V* (LAMPRECHT, 1951 a), which if the two segments *N—Fa* and *Le—V* are really situated in the same chromosome does not agree with the order *Fa—N—P. i.* (E)—*V—Le* indicated by the studies of NILSSON (1950). Since at present one cannot be quite sure that the difference between prime types E and A is confined only to a simple reciprocal translocation it is useless to discuss these discrepancies.

SUMMARY.

Meiosis has been studied in the PMC's of F_1 hybrids between some prime types (= segmental interchange lines) in *Pisum*.

The variety Extra Rapid is attributed to prime type D by NILSSON (1936), and to structural type 4 by SANSOME (1937). These two prime types are not identical (LAMM, 1949). SANSOME (1950) therefore seems to believe that two different translocations have arisen independently in the Extra Rapid variety. In the present paper I have pointed out that the structural type 4 is possibly identical with the prime type E (x-normal from N III). It must, however, be stressed that a definite proof of this suggestion has not been presented.

Chromosome 3, according to the numbers proposed by SANSOME (1937), is involved in prime type C (=Thibet 7), prime type D (=Extra Rapid N.) and prime type E (= x-normal from N III). In this chromosome the following order between the points of interchange (*P. i.*) and

the genes *St* and *B* has been found: —*P. i.* (D)—*St*—*P. i.* (E)—*B*—*P. i.* (C)—. The centromere is most probably situated within the *St*—*B* segment. In the translocation heterozygote between prime types D and A the interstitial segment, which contains the gene *St*, is long enough for chiasma formation.

In F_1 of prime type D × C five bivalents and a non-ring-forming configuration of four chromosomes have been observed. The gametic abortion was higher than in simple translocation heterozygotes. This behaviour could be explained by the occurrence of two interchanges involving two chromosomes, *i. e.* chromosome 1 and 3. The probable pachytene configuration is demonstrated by Fig. 8. *P. i.* (D) is situated in chromosome 1 between the genes *Gp* and *Fs*, and *P. i.* (C) is situated between the genes *Pa* and *R*. Until more investigations have been done I will, however, not claim for certain that the *Gp*—*Fs* and *Pa*—*R* segments really belong to the same chromosome. Several lines of evidence indicate that the centromere of chromosome 1 is situated in the neighbourhood of the gene *Gp*.

New genetical data are presented from the investigations made by Miss C. PELLEW concerning the prime type C. It has greatly facilitated the present investigations but it also contains some unexpected linkage values which ought to be further tested.

In prime type B (=HAMMARLUND's K-line) the point of interchange is inserted between the genes *Gp* and *A* (PELLEW, 1940). The former gene belongs to chromosome 1 of prime type A (= the normal structural type), the latter gene to chromosome 2. In chromosome 1 the differential segment between *P. i.* (B) and *P. i.* (D) seems to be too short for chiasma formation.

In prime type E one of the interchange chromosomes has a rather long interstitial segment. Rather reduced crossing-over values would therefore be expected for this segment in translocation heterozygotes.

An inversion bridge with a comparatively long fragment has been observed in some of the hybrids. I have suggested that the occurrence of this configuration may be explained by differential pairing between duplicated segments.

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EXPERIMENTAL STUDIES IN SCANDINAVIAN ALPINE PLANTS

II. ON THE ORIGIN OF THE GREENLANDIC SPECIES MELANDRIUM TRIFLORUM (R. BR.) J. VAHL

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IN Greenland three species of the genus *Melandrium* occur, which are all very closely related to each other, *viz. M. affine* J. VAHL, now named *M. furcatum* (RAF.) HULTÉN (HULTÉN, 1944, p. 702), *M. apetalum* (L.) FENZL. in LEDEB., and *M. triflorum* (R. BR.) J. VAHL. The two first-mentioned species also grow in Northern America, Asia, and Europe.

In a paper of 1923 OSTENFELD stated that *M. triflorum* is not so variable (*l. c.*, p. 175), while the other two species mentioned have a great number of varieties in the Arctic. In the thirties the three Danish botanists BÖCHER, GELTING, and SØRENSEN discussed the *Melandrium* species in question. SØRENSEN was the first to take up the subject (1933, p. 29). He wrote: »The Greenland plants have thus essential characters peculiar to *M. triflorum* s. str. more or less blended with characters typical of *M. apetalum*. — The material from the regions west of Greenland, Baffin Land and Arctic North America, belong to the same form group, even if here the specimens with uniflorous stems dominate. The species seems here on the whole to be less sharply demarcated from *M. apetalum*» (*l. c.*). According to the relations between *M. triflorum* and *affine*, SØRENSEN continues: »*M. triflorum* s. str. and *M. affine* are only distinguishable by the seed character usually employed, and by means of this character no natural division can be arrived at, since all transitional forms from tuberculate seeds without membranous margins to nearly smooth seeds with a fairly broad winged margin may be found.» Thus, according to SØRENSEN, all three species constitute a critical complex with transitional forms between the two extremes at each end of the series.

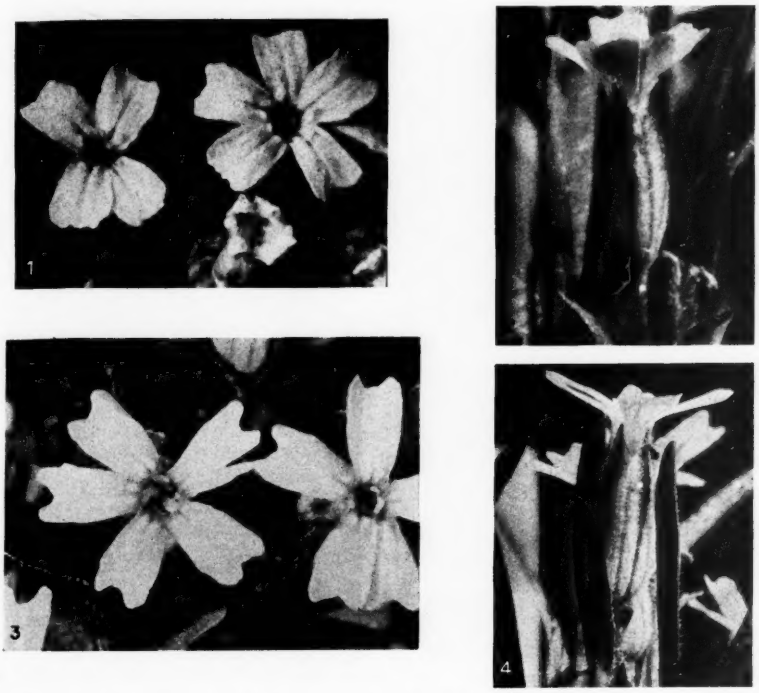
One year later GELTING took up the problem (1934, p. 48). He does not discuss the taxonomical position of *apetalum* but writes: »It is,

however, impossible to ascertain any certain relation between the outer morphological characters and the equipment of the seeds, and hence the two species, *M. triflorum* J. VAHL and *M. affine* J. VAHL, cannot be kept quite distinct. As will appear partly from the diagnosis, partly from a study of the forms referred by the various authors to *M. affine* J. VAHL, the latter species occupies an intermediate position between *M. triflorum* and *M. apetalum*. It may be assumed that at any rate part of the intermediate forms have originated by hybridization, even if this is difficult to ascertain» (*l. c.*).

At the end of the decade BÖCHER's work was published (1938). He finds the putting together of *M. affine* and *M. triflorum* under the name of *triflorum*, as was done by SØRENSEN and GELTING, too rigorous. »In any case a variety or subspecies should be set up to cover what has previously been understood as *M. affine*» (*l. c.*, p. 79). At the same time he points out as a matter of fact that *M. affine* has $n = 24$, while *M. triflorum* has $n = 36$ (BLACKBURN, 1930) and adds: »the number $n = 24$ for *M. affine* might perhaps agree with the assumption that this species is a hybrid between *M. apetalum* and *M. triflorum* (see GELTING, *l. c.*)» (*l. c.*).

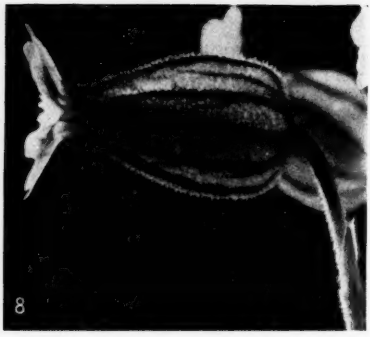
In 1949 the present writer published a paper on ecotypical differentiation in *Melandrium angustiflorum* (RUPRECHT) WALPERS. *M. angustiflorum* is a North European subspecies of *M. furcatum* (= *M. affine*). Two populations, one from Alta fjord in Northernmost Norway and another from Björkliden in North Sweden, were compared. The populations belonged to different ecotypes; when cultivated together at Uppsala they showed a difference in the time of flowering of about fourteen days, and besides that they deviated in some morphological characters (*l. c.*, p. 218). *M. angustiflorum* is a typical self-fertilizer, which in Scandinavia grows in a very restricted number of localities far away from each other (see HULTÉN, 1950, p. 191). For these reasons the writer interpreted the differences between the two populations as depending on a combination of isolation and homozygotization. At the same time it was pointed out that the two ecotypes deserved the rank of subspecies in as high a degree as the four subspecies of *Papaver radicatum* ROTTB. known from Scandinavia (*cf.* NORDHAGEN, 1931). The differences in the shape of the corollas and the calyces are clear from Figs. 1—4.

In 1948 the Alta as well as the Björkliden ecotype of *M. angustiflorum* was crossed with *M. apetalum* and *M. rubrum*. In the last combination no seeds at all were obtained, but in one cross between the



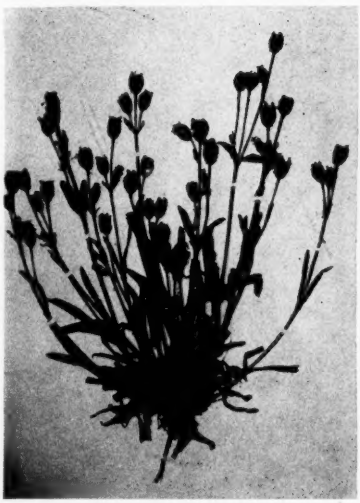
Figs. 1—4. — 1. To the left, upper row: *Melandrium angustiflorum*, the mountain ecotype from Abisko. — 2. To the right, upper row: the same as No. 1. — 3. Below, to the left: *M. angustiflorum*, the alpine lowland ecotype from Alta fjord. — 4. Below, to the right: the same as No. 3.

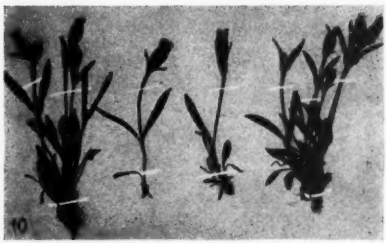
Björkliden ecotype of *angustiflorum* and an *apetalum* biotype from the Dovre Mountains in Central Norway some seeds were formed. When the seedlings were tested in the summer of 1949, no differences could be observed between the supposed hybrid plants and seedlings from *M. apetalum*, and the cross was accordingly interpreted as having been unsuccessful. In the summer of 1950, however, it appeared obvious that the cross had been successful. Five plants flowered that summer, which all looked morphologically intermediate between *M. angustiflorum* and *M. apetalum* (Figs. 5—8). This intermediacy was especially clear as to the shape of the corollas and the calyces. The hybrid had an inflated calyx, which is characteristic of *apetalum*, at the same time as its protruding corollas were typical of *angustiflorum*. A quite new



Figs. 5—8. The hybrid between *M. angustiflorum* and *M. apetalum*, $2n = 36$.

plant had arisen, which had not earlier been seen in Scandinavia. Outside Scandinavia *M. triflorum* is that species of the genus which taxonomically comes nearest to the new hybrid (Figs. 9—11). *Triflorum* has an *apetalum* calyx (*cf.* Figs. 13, and 12 and 14), and at the same time its corollas are more protrudent than in *apetalum* but less so than in *angustiflorum*. The hybrid is taller than *angustiflorum*; on an average the five plants are 20,73 cm. high, while the corresponding figure for the Björkliden ecotype is 16,7 cm. and that for the Alta ecotype 14,8 cm. The mean of the leaf-length in the hybrid plants is 4,52 cm. and that of the leaf-breadth 0,723 cm., while *angustiflorum* from Björkliden has leaves averaging 2,84 cm. long and 0,56 cm. broad. The corresponding figures in the Alta ecotype are 2,7 and 0,29, respectively. The relation leaf-length/leaf-breadth is 6,33 in the hybrid, while it is 5,07 in the Björkliden ecotype and 8,97 in the Alta ecotype.

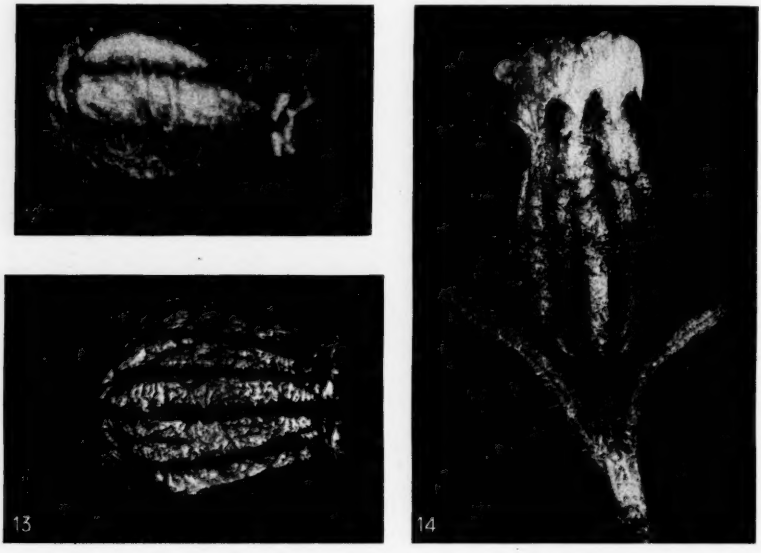




Figs. 9—11. — 9. To the left: *M. triflorum*, specimen with ripe capsules from Scoresby Sound, East Greenland. — 10. Upper, to the right: Young specimens of *M. triflorum* from Cape Stewart. — 11. Below, to the right: *M. triflorum*, broad-leaved specimens from Valrossön.

No measurements were made in the Dovre biotype of *apetalum* when it flowered in cultivation, but it is obvious from dried material that this species would have given somewhat higher figures for length and breadth of the leaves than the hybrid, while the plant height was about the same in both.

The figures for the leaf-length and the leaf-breadth in the hybrid correspond fairly well with the situation in *M. triflorum*, according to the material available in the Museum of Natural History in Stockholm. It is evident, however, that *triflorum* varies a great deal in these respects. The same applies to the number of flowers in the cymes of *triflorum*, which varies between one and three. In the hybrid the same variation appears, but most branches have only one or two flowers. The pedicels are much longer in the hybrid than in *triflorum*, but this circumstance probably depends on differences in the length of day in Greenland and at Uppsala. The hybrid plants flowered at Uppsala in a greenhouse during the end of April and the beginning of May, whereas in Greenland, according to GELTING and SØRENSEN (*l. c.*), *triflorum* flowers in June, when the sun never sets at the latitudes where the species occurs

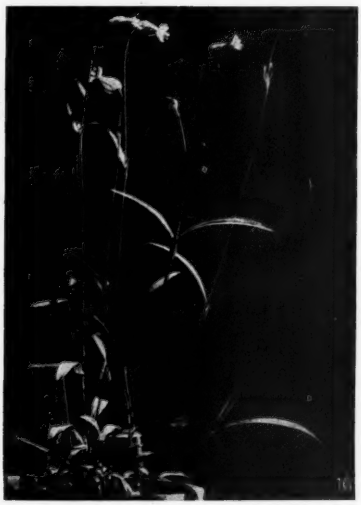


Figs. 12—14. — 12. Uppermost, to the left: ripe capsule of *M. triflorum* (taken from plant Fig. 9). — 13. Below, to the left: old capsule from *M. apetalum* (dried specimen from Dovre). — 14. To the right: Young flower from *M. triflorum* (taken from one of the plants on sheet Fig. 10).

on that island. In this connection attention should be called to the paper of BÖCHER and LARSEN in 1950, which has a picture of *triflorum*, cultivated in Copenhagen, that has long branches with one or two flowers just as the Uppsala hybrid (*l. c.*, p. 17), and shows great morphological similarities to the actual hybrid (*cf.* Figs. 15 and 16).

In order to prove whether GELTING and BÖCHER's hypothesis that *M. furcatum* should be a hybrid between *M. apetalum* and *M. triflorum* is valid, we must first examine the chromosome numbers of the three species. They are:

Melandrium furcatum (as *M. affine*)	$2n = 48$	BLACKBURN, 1930
subsp. *angustiflorum*	$2n = 48$	NYGREN, 1949
Melandrium apetalum	$2n = 24$	BLACKBURN, 1930
		LÖVE and LÖVE, 1942
		NYGREN, 1949



Figs. 15—16. — 15. *M. triflorum*, cultivated in Copenhagen, to the left. After BÖCHER and LARSEN, 1950, p. 17. — 16. The hybrid between *M. angustiflorum* and *M. apetalum*, to the right. — *M. triflorum* has $2n = 72$, the hybrid $2n = 36$.

Melandrium triflorum	$2n = 72$	BLACKBURN, 1930 BÖCHER and LARSEN, 1950

The chromosomes of *Melandrium furcatum* and *triflorum* were counted by BLACKBURN on Greenlandic material. *Angustiflorum* has been determined as to chromosome number by the present writer in the ecotypes just mentioned as well as *apetalum* from six different Scandinavian localities. By BÖCHER and LARSEN's verification of BLACKBURN's number for *M. triflorum* the chromosome numbers in all three species can be assumed to have been satisfactorily checked. Thus, it is evident that a cross between *apetalum* and *triflorum* really would give a hybrid with 48 chromosomes somatically, just as *furcatum* has, but the author shares BÖCHER's apprehension as to the fertility of such a hybrid (*l. c.*, p. 79). On the other hand, the hybrid between *apetalum* and *furcatum* should have $2n = 36$, *i. e.* the number that the actual hybrid produced at Uppsala has. If this hybrid is chromosome-doubled, an amphidiploid with $2n = 72$ will be obtained. Such an amphidiploid would probably show a good seed setting as well as a fairly great

variability in the offspring, just as is characteristic of *M. triflorum*, and for these reasons the author considers *triflorum* to be an amphidiploid between *M. apetalum* and *M. furcatum*, which by mere chance has been formed only in Greenland though both parents occur together in other parts of the Arctic.

The amphidiploid has not yet been produced experimentally, because all five hybrid plants were completely sterile in 1950 and, thus, no seeds were obtained. *M. apetalum* as well as *M. angustiflorum* generally flowers two successive years in cultivation, however, and for that reason there is some hope that the five plants will survive the winter of 1951 so that more experiments can be made with them.

The problem of *triflorum* is not the only one in arctic *Melandrium*, however. In the Flora of Alaska and Yukon by HULTÉN as well as in the Flora URSS there are more complex groups, the origin of which cannot be solved as long as the present political situation makes it impossible to obtain material of any kind from the great territories in Northeastern Europe and Northern Asia.

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LOCALIZED CHROMOSOME BREAKAGE IN VICIA FABA

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(A preliminary note)

IT has been shown recently that various mutagenic substances have the ability of breaking the chromosomes of *Vicia faba*, preferably in certain chromosome regions. FORD (1949) and LOVELESS and REVELL (1949) found that nitrogen mustard induced chromosome fragmentation more frequently in the *st* chromosomes than would have been expected if the breaks occurred at random. The breaks in these cases were probably situated close to, or within, the heterochromatic knobs of the *st* chromosome. LEVAN and LOTFY (1950), studying the spontaneous chromosome breaks occurring after starting the germination under anaerobic conditions, found a similar predilection for the fragmentation to occur in the *st* chromosomes, although the points of breaking were somewhat more proximal in this case. DARLINGTON and MCLEISH (1951) treated *Vicia faba* with the growth regulator maleic hydrazide. They observed that the chromosome breaks, frequently occurring, were confined to two distinct heterochromatic regions: the proximal part of the nucleolar arm of the *m* chromosome, and the proximal part of the long arm of one of the *st* chromosomes.

KIHLMAN (1951) synthesized several purine derivatives and tested them as to their radiomimetic activity in *Allium Cepa*. It was found that two groups of substances could be distinguished: one group (of the type 8-ethoxycaffeine, EOC) was able to penetrate through the nuclear membrane into the resting nuclei, where structural changes were induced; the second group (of the type 1.3.7.9-tetramethyluric acid, TMU) did not penetrate directly through the nuclear membrane. The substances of this second group entered the resting nuclei through the telophases and thereafter were able to induce chromosome fragmentations.

In the present investigation KIHLMAN treated roots of *Vicia faba* seedlings, germinated under aerobic conditions in soil, with the two type compounds EOC and TMU. The chromosome responses to the treatments were studied by us conjointly. The following treatments were given: 7,5 mmol/l EOC for 6 hours at a temperature of 10 C., thereafter

recovery for 24 or 48 hours at 20 C.; 5 mmol/l TMU for 24 hours at 20 C., followed by recovery for 24 hours at the same temperature. Treatments at these temperatures were known to give maximal frequency of chromosome changes in *Allium*. A treatment with EOC at 20 centigrades gave qualitatively the same result as the above treatment, only a smaller frequency of structural changes. Slides were prepared according to KIHLMAN (1951). Some slides were made according to the oxiquinoline technique.

It was immediately found that the EOC induced an abundance of fragments of a very specific type. It was soon realized that these fragments in the predominant number of cases consisted of the satellite part of the *m* chromosomes. In many cases it was seen directly that the anaphase groups contained the centric part of the *m* chromosome deprived of its satellite. Within one chromosome, as a rule, both chromatids behaved in a similar way, the chromosome break occurring on the same level in both chromatids. Sister reunion was the rule in the acentric fragments but seldom occurred in the centric part of the chromosome. Other types of fragments, however, were also encountered. In the following two slides from the EOC treatments are analysed, the acentric fragments found being classified into three groups: (I) satellite fragment, (II) big fragments, and (III) small fragments.

	Slide number 1	2
one satellite fragment	4^1	—
two » fragments	67^5	115^2
three » »	2	—
four » »	17^2	15^1
one big fragment	7^2	—
» » » + two satellite fragments	4^2	1
» » » + four » »	2	1
» » » + one small fragment	—	1
two big fragments	2^1	1^1
» » » + two satellite fragments	4	—
one small fragment	7^3	1^1
» » » + two satellite fragments	1^1	—
two » fragments + » » »	2^1	1^1
no fragment	85^2	166^1
Total	204^{20}	302^7

The exponential numbers in the above table show the number of cells with one, or in exceptional cases, two bridges, indicating sister reunion in the centric fragments. Such bridges are much rarer than, for instance,

in DARLINGTON and MCLEISH's experiments. Since the translocation point is located within the attachment thread, however, the resulting bridge may be expected to be little resistant to the anaphase pulling. In some cases a drawn-out thread could be seen between the two arms in question. Also the thread connecting the acentric satellite fragments was sometimes pulled out very far.

As seen, the two slides analysed behave somewhat differently. In slide 1, 58 % of the cells contain fragments, each cell having 0,75 breaks. In slide 2, the corresponding values are 45 % and 0,52. What is more interesting is the frequency of breaks within the attachment thread of the satellite: such breaks had occurred in 124 cases out of 153 in slide 1, and in 149 of 156 cases in slide 2 (81 and 96 %, respectively). Thus, the root tip with the lower absolute frequency of breaks had given the highest frequency of fragmented satellites.

The appearance of the present structural changes is seen from Fig. 1 *a* and *b*. In both cases one *m* chromosome has been broken at the attachment thread of the satellite, sister reunion having occurred in both the centric and the acentric fragments. Fig. 1 *c* shows a number of fragments from these treatments. Most of them are satellites, although a few deviating ones are figured to the right in Fig. 1 *c*. Very often two extremely small Feulgen-positive granules were seen on the attachment thread between the two connected satellites. These granules may be found now and then on the attachment thread also *in situ* in the unchanged *m* chromosome. These granules seem to be a typical feature of the attachment thread; their occurrence may make it possible to locate the point of translocation somewhat more closely. They are not always found, however, so it cannot be said for certain whether the breaks always occur at the same point, *viz.* proximally of the granules. They have never been seen in the few reunion bridges studied, so it is probable that the greatest part of the attachment thread goes with the satellite fragment.

If the time of treatment is longer, for instance nine hours, fragments of all types are obtained, the relative frequency of satellite fragments decreasing. Often very strong changes are met with, entire metaphase plates being »pulverized». With the concentration of EOC employed, longer exposures than twelve hours almost regularly kill the roots.

Also TMU induced a considerable frequency of structural changes. These changes seem to be somewhat less regular than in the case of EOC. A pronounced erosion of certain chromosome regions was often

seen. This erosion gave rise to wide secondary constrictions or exaggerated ones already present. Since the constrictions were often seen to cut off chromosome segments of about the same size as the satellites, it was often impossible to identify free fragments as satellites, if the entire chromosome complement could not be analysed. In many cases

Fig. 1. *Vicia faba.* Somatic anaphase chromosomes. — *a*, *b*: EOC treatment; one *m* chromosome fragmented at the satellite connection fibre, reunion both in the centric and the acentric fragments. — *b*: treated with oxiquinoline. — *c*: EOC treatment; the acentric fragments from 25 anaphases; in most cases the fragments consist of the satellite. — *d*: germination started under partly anaerobic conditions: acentric fragments from 25 anaphases; the fragments have a different type from those induced by EOC.

it was made certain that satellite-like fragments were not satellites, both *m* chromosomes of the same cell being found to have their satellites intact. In the same manner often more than four satellite-like fragments were present in one cell. In many cases, however, there was no doubt that satellites formed free fragments. In addition, there occurred all types of fragments from small minute ones to very big ones. Divided

into similar size classes as in the case of EOC, the various types of fragments recorded in two slides had the following frequencies:

	Slide number 1	2
two satellite-like fragments	11[1]	8
four » » »	4[1]	—
five » » »	—	1
six » » »	2	2
nine » » »	1	—
one big fragment	4	—
» » » + two satellite-like fragments	1	—
» » » + four » » »	—	1
two big fragments	2	2
» » » + two satellite-like fragments	—	2
one small fragment + one » » fragment	1	—
two » fragments	2	1
» » » + four satellite-like fragments	1	—
» » » + five » » »	—	1
no fragment	90[5]	69
Total	119[7]	87

The frequency of cells with fragments is decidedly lower than in EOC treatment, *viz.* 24 and 21 %, respectively. Although undoubtedly many of the satellite-like fragments recorded above are really free satellites, it is not possible to make any estimation of their frequency in relation to other types occurring.

In order to obtain a comparison with the fragments occurring after germination in partially anaerobic conditions, one root tip thus treated was analysed. This root came from a seed which had been submersed in water for 24 hours before germination and afterwards kept in moist filter paper for 48 hours before fixation. Structural disturbances were abundant in this root. 40 out of 106 anaphases had various numbers of fragments. Among these fragments very big ones predominated, just as was found by LEVAN and LOTFY (1950). Often the very large fragments were double, the two halves showing relational coiling. This situation, as well as the frequent occurrence of micronuclei and, within the anaphase groups, of small centric fragments or pulled-out bridges of such diminutive chromosomes indicated that the fragmentation had taken place at a preceding mitosis. It is of interest to note that sometimes the acentric fragments may behave as the ordinary chromosomes, synchronizing their contraction and spiralization with that of the centric chromosomes, while in other cases the acentric fragments go into the

resting stage without the possibility of re-entering prophase. Maybe this difference is caused by the nuclear environment, fragments which happen to get into the main nuclei behaving as normal chromosomes, while fragments forming micronuclei have lost the possibility of re-entering mitosis.

In this partly anaerobic treatment there were only found 5 out of 40 recorded cases where the fragments on account of their size could be satellites; all other fragments occurring were quite different in type. Thus, there is no doubt that this type of fragmentation is distinctly different from that induced by the two purine derivatives of the present investigation. Some acentric fragments, picked out at random, are pictured in Fig. 1 *d*.

A control, germinated in soil, was also closely studied. It did not contain any fragments. Thus the observation of LEVAN and LOTFY (1950) that submersion in water at the start of the germination predisposes to chromosome fragmentation is confirmed by the present study.

The main interest of this investigation is centred on the fact that one certain part of the entire chromosome complement of *Vicia faba* is especially liable to breakage under influence of the chemicals employed. Since other chemicals have been reported to attack, preferably at least, other distinct chromosome regions, it seems that a work along these lines will give valuable information about the differential organization of the chromosomes. It should be mentioned that pyrogallol in various plants, among them *Vicia sativa*, according to THERMAN-SUOMALAINEN (1949) »in the first place attacks the chromosomes at the hiata created by the secondary constrictions» (*l. c.*, p. 251). It will be especially interesting to investigate whether this tendency to fragmentation of the attachment thread by purines is a general feature common to many plants, or if *Vicia faba* is a special case. DARLINGTON and MCLEISH (1951), for instance, were not able to break heterochromatic regions of other plants than *Vicia faba* with maleic hydrazide.

SUMMARY.

Root tips of *Vicia faba* were treated with solutions of two purine derivatives known to induce structural chromosome changes in *Allium Cepa*. Both substances effected a pronounced chromosome fragmentation. Especially 8-ethoxycaffeine had the property of predominantly breaking the attachment thread of the satellite in the *m* chromosomes of *Vicia faba*.

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THE MUTANT "MICRO" IN PISUM

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INTRODUCTION.

THE mutant »micro» has been described by Professor J. RASMUSSON, who first found it in F_8 of a cross between pure lines from the pea varieties Fürst Bismarck and Grå dvärg (RASMUSSON, 1938). The name given to it is very appropriate as the »micro» type is an extremely small dwarf, normally only a few inches high and capable of producing only a few pods. RASMUSSON (*l. c.*) gives figures which show that all parts of the plant are smaller in the mutant than in the motherline from which it originates. Apart from its small dimensions the micro type is normal in every respect although, not surprisingly, its viability is somewhat reduced.

The F_8 family in which micro first appeared consisted of 23 normal plants and 7 micro. RASMUSSON assumed that micro differs from normal only in a single recessive gene for which he used the symbol *lm*. He has kindly handed over to me material of the »micro» mutant together with several pure pea lines for investigation of the relation between *lm* and other height factors in *Pisum* and of the linkage relations of the new gene.

MATERIAL.

A list of the material used for the crosses is given below. In this list is also shown the genotypes, as far as they are known, of the parents with regard to 18 genes which have been studied in the investigation.

No. 1 is a pure line descending from the original micro mutant. When referring to this pure line the word »Micro» will be spelt with a capital M, when referring to the type it will be spelt with a small m.

Nos. 2 to 10 are lines which have been grown for several generations and which have always been found homozygous for the genes mentioned in the list.

Nos. 11 to 14, finally, have been taken straight from segregating families and have been used as cross parents without any previous test for homozygosity.

1. Micro	*lm le St Tl A B K I R S pl p v Wa Wb Wlo*	Cy_1	Cy_2
2. de Winton	*Lm Le st Tl A b k I R s pl P V Wa wb wlo*	—	—
3. Witham Wonder	*Lm le St Tl a B K i r S pl P V Wa Wb Wlo*	Cy_1	$cy^c{}_2$
4. Acacia	*Lm le St tl a B K I r S pl P V Wa Wb Wlo*	Cy_1	$cy^s{}_2$
5. Fürst Bismarck	*Lm Le St Tl a — K I R S pl p V Wa Wb Wlo*	Cy_1	Cy_2
6. Black-eyed Lincoln Blue	*Lm Le St Tl a B K i R S Pl P V Wa Wb Wlo*	—	—
7. Chiando	*Lm Le St Tl a — K I R S Pl P V Wa Wb Wlo*	—	—
8. Cryptodwarf Lamm	*Lm le St Tl a B K i R S pl P V wa Wb wlo*	cy_1	$cy^c{}_2$
9. Slender	*Lm le St Tl A B K I R S pl P V Wa Wb Wlo*	cy_1	$cy^s{}_2$
10. Acacia microdwarf	*lm le St tl a B K I r S pl p V Wa Wb Wlo*	cy_1	$cy^s{}_2$
11. microtall	*lm Le st Tl A B k I R s pl p V Wa wb Wlo*	—	—
12. *wlo*-microdwarf	*lm le St Tl A B K i R S pl P V* $\frac{Wa}{wa}$ *Wb wlo*	—	—
13. microdwarf from cross 2	*lm le St Tl A B K I r S pl p v Wa Wb Wlo*	Cy_1	Cy_2 $cy^c{}_2$
14. microdwarf from cross 3	*lm le St Tl A B K I R S pl P V Wa Wb Wlo*	Cy_1	Cy_2

The results to be reported have been obtained from the following 14 crosses.

1. de Winton × Micro.
2. Witham Wonder × Micro.
3. Acacia × Micro.
4. Witham Wonder × microtall.
5. Cryptodwarf Lamm × microdwarf from cross 2.

5a. Cryptodwarf Lamm × microdwarf from cross 3.

6. Black-eyed Lincoln Blue × Acacia microdwarf.
7. Chiando × Acacia microdwarf.
8. Black-eyed Lincoln Blue × *wlo* microdwarf.
9. Cryptodwarf Lamm × Acacia.
10. Cryptodwarf Lamm × Fürst Bismarck.
11. Fürst Bismarck × Acacia.
12. Slender × Micro.
13. Fürst Bismarck × Micro.

CYTOLOGICAL OBSERVATIONS.

A cytological investigation was carried out in order to ascertain whether the micro-character was associated with any chromosome abnormalities.

For the purpose of these studies root tips were fixed in Navashin's fixative and pollen mother cells in Navashin after pre-treatment for one minute in Carnoy. All fixations were embedded in paraffin wax. New-

ton's crystal violet method was used for staining. Pollen samples were mounted in a mixture of equal parts of acetocarmine and glycerine in the ordinary way.

In root tips of the Micro-line 14 chromosomes were found in all dividing cells and the chromosome complement appeared completely normal.

Meiosis was studied in F_1 plants of the following three crosses:

Fürst Bismarck × Micro.

Black-eyed Lincoln Blue × *wlo* microdwarf.

Chiando × Acacia microdwarf.

In the first two crosses chromosome pairing was perfectly normal.

Two buds with dividing P.M.C. were found in the third cross. In one of the buds the chromosomes showed normal pairing, in the other certain abnormalities were observed, which will be described below.

Pollen samples from the three crosses all showed good pollen fertility.

In spite of the case of abnormal meiosis encountered in one bud of the cross Chiando × Acacia microdwarf it is clear from the above results that the dwarf character of the microdwarf type is not, as a rule, connected with any cytological peculiarities.

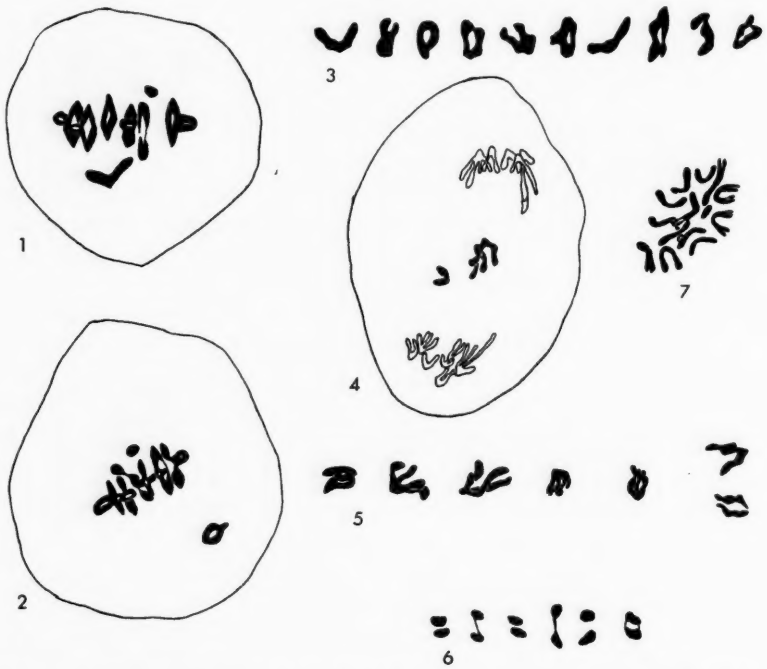
ABNORMAL MEIOSIS IN F_1 CHIANDO × ACACIA MICRODWARF.

In one bud from this cross meiosis was irregular in all dividing pollen mother cells. At metaphase 1 (Figs. 1 and 2) six bivalents were found arranged on the equator in the normal way. Outside the group formed by these six bivalents there were a small univalent and a structure which was at first interpreted as a bivalent which had failed to orientate itself on the spindle. But its appearance differed from that of other bivalents and after a closer study it was concluded that this structure was a univalent in which the two arms usually pair with one another. Fig. 3 illustrates its appearance in 10 different cells. Sometimes it assumed the shape of a rod but usually it was more or less ring-shaped. Out of 210 cells it was found to be ring-shaped in 185 (88,1 %) and rod-shaped in 25 (11,9 %). This indicates that the pairing segment was of considerable size. This univalent was often similar in appearance to the co-univalent found by HÅKANSSON in *Triticum* (HÅKANSSON, 1933).

The small univalent was of constant size and discoid in appearance. The small and the big univalents were sometimes seen very close

together and in a few cases they appeared to be associated with each other.

At first anaphase the univalents sometimes reached the poles before the normal chromosomes but often one or both were lagging



Figs. 1—6: Abnormal meiosis in F_1 Chiando × Acacia microdwarf. — 1—2: Metaphase 1. — 3: Big univalent at metaphase 1 in 10 cells. — 4: Anaphase 1 with big and small univalents lagging. — 5: Big univalent at anaphase 1 in 6 cells; misdivision on extreme right. — 6: Dividing small univalents. — Fig. 7: Chromosomes from somatic cell.

(Fig. 4). As a rule they did not divide at first anaphase but some cases of division were observed. The big univalent at this stage consisted of four arms of approximately equal length (Fig. 5). In one cell a form of misdivision was observed, two arms being left on the equator, acentric or perhaps with a telocentric centromere each (*cf.* DARLINGTON, 1939, Fig. 7 L and MÜNTZING, 1944, Fig. 24). Owing to the small size of the other univalent the position of its centromere could not be deduced from

its appearance in those cases when it was observed to divide at anaphase (Fig. 6). No instance was observed where the laggards failed to be included in the daughter nuclei but observations on this point were rendered difficult by the presence of extra-nuclear nucleoli which are characteristic of *Pisum* (HÅKANSSON and LEVAN, 1942). After staining with crystal violet it is not possible to distinguish between chromatin and nucleolar matter.

Unfortunately the slide did not contain any stages of the second division. No root tips were fixed of this F_1, but divisions were seen in somatic tissue of the bud with abnormal meiosis. In three cells the normal chromosome number 14 was observed. The plates were not so good as those usually found in root tips and although they permitted determination of the chromosome number they did not allow a detailed study of the morphology of the chromosomes. In one of the cells, however, a very small chromosome could be seen (Fig. 7), presumably that which formed the small univalent at meiosis.

The lack of observations on the second division and on somatic chromosomes makes it difficult to decide with certainty what structural change has taken place in this case.

The explanation of the observed abnormalities may be found in the translocation of the greater part of the long arm of a chromosome with subterminal centromere to the short arm of the homologous chromosome. Such a translocation would give a big chromosome with submedian centromere and a small chromosome which could have a median, submedian or subterminal centromere according to where the breakage of the long arm had taken place. There is nothing in the facts observed at meiosis that contradicts this explanation. The arms of the big chromosome would be expected to pair and thus give a ring-shaped univalent. The small chromosome would be expected to pair occasionally with its homologous part in the big chromosome. An association of the small and the big univalents has actually been observed in a few cases. This explanation also accounts for the fact that the chromosome number 14 was found in somatic cells of the bud with abnormal meiosis.

It is not possible to explain the facts in a simple way as due to a structural change of any kind in the parents. In another bud from the same F_1, meiosis was found to be completely normal. This may mean that one of the F_1 plants was a chimaera for the structural change, but unfortunately it is not known whether the two buds came from the same plant. It is however difficult to escape the conclusion that the change had taken place in a somatic cell of an F_1 plant.

Plants chimaerical for a structural change have been reported by NAVASHIN (1931) in *Crepis* and by DARLINGTON (1933) in *Secale*. NAVASHIN describes, amongst other cases, one in which »the proximal fragment of one of the A-chromosomes has become a detached, diminished autonomous chromosome, the rest of this A has fused with the proximal end of the other, otherwise unaltered A to give a V-shaped structure». This case appears to be very similar to the one described here.

GENETICAL INVESTIGATIONS.

METHODS.

In all approximately 14.000 plants have been grown and classified. The investigation was made rather difficult by the fact that the »micro»-plants had a very low viability when grown in the field. Many of these dwarfs died before flowering and very few survived long enough to produce well developed pods and seeds. A method of growing the microdwarfs to maturity had to be found before the investigations could be started. They could be distinguished from the normal plants at a very early stage and attempts were made to transplant the »micro» plants to pots or boxes which were placed in a greenhouse or in cold frames. This method was reasonably satisfactory but the microdwarfs had to be handled very carefully both at transplanting and afterwards and even so they did not develop really well. Often the pods were poorly developed and pod characters difficult to classify. Better results were obtained through sowing all the seeds of segregating families in a suitable seed compost in cardboard cartons. The seedlings were later transplanted to the field, care being taken not to disturb the roots. When treated in this way the microdwarfs developed quite satisfactorily, in fact they seemed to stand the transplanting operation better than the normal plants which often suffered a check when weather conditions were unfavourable.

In the statistical treatment of the data the χ^2 method has been used in testing for deviations from expected ratios, for linkage and for heterogeneity between crosses or between families within crosses. Where the segregations were disturbed the BRANDT and SCHNEDECOR method (MATHER, 1938, Chapter II) was employed in testing for heterogeneity in single gene ratios and the following formula was used for calculation of the linkage χ^2 (MATHER, 1946).

$$\chi^2_L = \frac{(a_1 - l_1 \times a_2 - l_2 \times a_3 + l_1 \times l_2 \times a_4)^2}{n \times l_1 \times l_2}$$

l_1 and l_2 are the ratios between dominants and recessives for the two single gene segregations. This formula gives the same results as the contingency table method but it has the advantage of allowing calculation of a heterogeneity χ^2 provided that the different sets of data are homogeneous with regard to the value for l_1 and l_2. When only one of the gene ratios showed a deviation from 3 : 1 it was adjusted in the way described, but the other segregation was allowed to take the expected ratio of 3 : 1 which leads to the formula:

$$\chi^2_L = \frac{(a_1 - 3 \times a_2 - l \times a_3 + 3 \times l \times a_4)^2}{3 \times l \times n}$$

These formulae have been used only in those cases where one or both single gene ratios show a deviation from 3 : 1 which corresponds to a P-value of 0,05 or less.

Different levels of significance of the χ^2 values have been indicated in the tables in the following manner:

* χ^2 corresponding to a P-value between 0,05 and 0,01
** » » » » » » 0,01 » 0,001
*** » » » » » smaller than 0,001.

The method of maximum likelihood has generally been used in calculating recombination values but the product formula has been preferred in cases of disturbed segregations. Standard errors for recombination values and χ^2 for heterogeneity between different sets of linkage data have been calculated in accordance with the methods given by MATHER (1938).

OTHER HEIGHT FACTORS IN PISUM.

Three loci affecting plant height in *Pisum* have long been known, namely *Le*, Cy_1 and Cy_2. *Le* gives tall plants with many long internodes, *le* gives low plants or dwarfs with few short internodes. (In order to avoid confusion with the microdwarfs this type will here be referred to as »low».) Cy_1 and Cy_2 are complementary genes and there are three alleles of the Cy_2 locus known, namely Cy_2, cy_2^c, and cy_2^s (LAMM, 1937). Plants which carry one or both of the dominant genes Cy_1 and Cy_2 are normal, those carrying $cy_1\ cy_1\ cy_2^c\ cy_2^c$ are cryptodwarfs, and those with $cy_1\ cy_1\ cy_2^s\ cy_2^s$ are slenders.

The F_8 family in which the micro-mutant first appeared was homozygous for the recessive *le* and did not contain any cryptodwarfs or slenders. The »Micro»-line used in this investigation has been found to have the following genotype with regard to the genes affecting height: *lm lm, le le, Cy_1 Cy_1, Cy_2 Cy_2*.

INHERITANCE OF THE »MICRO»-CHARACTER AND INTERACTIONS WITH OTHER HEIGHT TYPES.

As has already been mentioned, RASMUSSON's data suggest that the micro-character is conditioned by a single recessive gene, *lm*. Results of crosses involving the micro-character and other height characters confirm this conclusion. These results are presented in Tables 1 and 2. The total of all segregating F_2- and F_3-families gives a ratio of 9711 normal : 3003 »micro», *i. e.* a slight, although significant deficiency of the recessive ($\chi^2 = 12{,}920^{***}$; $P = 0{,}001$). This deficiency is mainly caused by the F_3 families originating from low plants in F_2 of cross No. 1. The reason for this discrepancy will be explained later. The heterogeneity χ^2 for all those 235 F_2- and F_3-families which segregated *Lm—lm* amounts to 236,118 which indicates that the homogeneity of the data is satisfactory.

The ratio between constant and segregating F_3 families is generally in good agreement with expectation. An exception is the large excess of segregating families in cross No. 5, Cryptodwarf Lamm × microdwarf. This will be discussed later.

It will be noted that the percentage of germination in F_2 of cross No. 2 Witham Wonder × Micro is very low (55,7 %). This is presumably due to the fact that the seed used was several years old. It does not appear to have had any influence on the segregation normal : micro.

The difference between the two types low and microdwarf is seen in Fig. 8. Although classification usually is easy, poorly developed specimens of the normal type occasionally cause some difficulty. In most cases, however, the microdwarfs have thinner stems and on the whole a more frail appearance than under-developed low plants. In doubtful cases the plants have to be tested in the following generation. That a certain caution is necessary when classifying is shown by the fact that one year a plant exactly resembling a microdwarf appeared in the pure line Acacia. The plant survived on the field and produced a few pods and a few seeds. Its offspring the following year could not, however, be distinguished from the normal Acacia line.

The F_1 generation in the crosses between low and microdwarf was

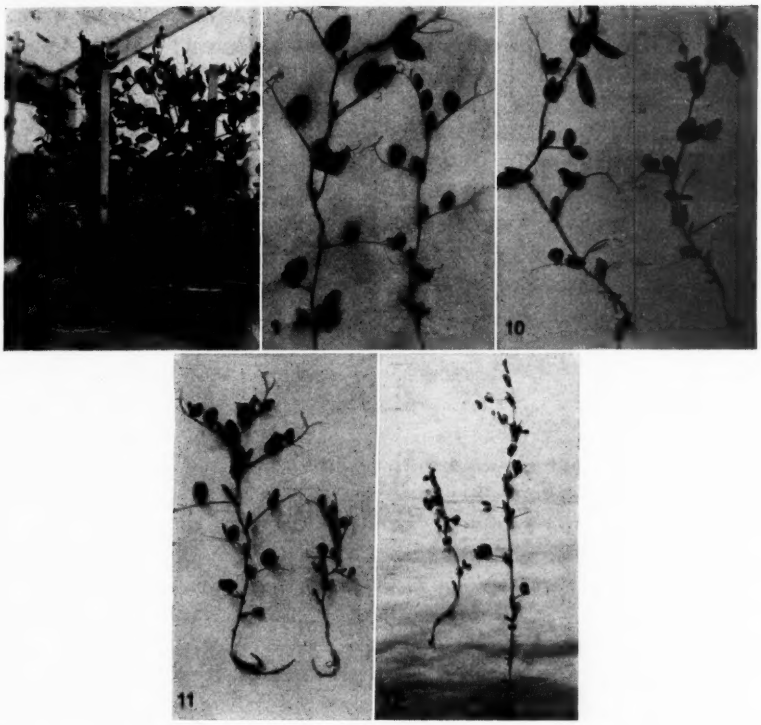


Fig. 8: Microdwarfs in front row, low plants in back row. Grown in greenhouse. — Fig. 9: Microtall (right) and tall (left). Young plants. — Fig. 10: Two fully developed microtalls grown in pots. — Fig. 11: Micro-cryptodwarf (right) and cryptodwarf (left). Young plants. — Fig. 12: Microslender (left) and slender (right). Young plants.

of the same height as the low parent. The normal type appeared to be completely dominant.

The relation between *Le* and *Lm* could be studied in crosses Nos. 1, 6, 7 and 8 in which one parent is tall and the other microdwarf. The F_1's of these crosses could not be distinguished from the tall parent in regard to height. In F_2 four types appeared: tall, low, microtall and microdwarf. The new type microtall is much taller than the ordinary microdwarf from which it differs through having more and longer internodes. In these experiments, well developed microtalls have at maturity usually reached a height of 20—40 cm and microdwarfs a height of 5—15 cm. Microtall differs from tall in the same way as microdwarf differs from

TABLE 1. *F_2 analysis.*

Genotypes of parents	Cross No.	Number of families	Genes		Number of plants							% classified plants	χ^2 for deviation			χ^2 for heterogeneity		
									Total		Grand total							
			A	*B*	*AB*	*Ab*	*aB*	*ab*	*Lm*	*lm*			*Aa*	*Bb*	Linkage	*Aa*	*Bb*	Linkage
Lm Lm le le × *lm lm le le*	2	5	*Lm*						245	71	316	55,7	1,080			1,484		
» »	3	8	*Lm*						540	181	721	81,0	0,004			9,498		
Lm Lm Le Le × *lm lm le le*	1	2	*Lm*	*Le*	341	103	93	30	444	123	567	95,9	3,307	0,720	0,104	0,001	0,005	0,091
» »	6	1	*Lm*	*Le*	153	54	44	14	207	58	265	83,8	1,370	0,062	0,094	—	—	—
» »	7	1	*Lm*	*Le*	92	41	20	18	133	38	171	88,6	0,704	8,236**	3,579	—	—	—
» »	8	5	*Lm*	*Le*	179	45	54	19	224	73	297	81,0	0,028	1,887	1,051	4,957	9,542*	1,897
Lm Lm le le × *lm lm Le Le*	4	2	*Lm*	*Le*	186	66	57	31	252	88	340	94,4	0,141	2,259	3,012	0,278	0,171	0,168
Lm Lm $cy_1cy_1cy_2^cCy_2^c$ × *lm lm* $Cy_1Cy_1Cy_2Cy_2$	5,5a [1]	5	*Lm*	$Cy_1 + Cy_2$	565	32	189	12	597	201	798	99,5	0,015	0,738		3,335	1,533	
Lm Lm $cy_1cy_1cy_2^cCy_2^c$ × *lm lm* $Cy_1Cy_1cy_2^cCy_2^c$	5 [2]	1	*Lm*	Cy_1	52	14	18	9	66	27	93	97,8	0,806	0,004		—	—	—
Lm Lm $cy_1cy_1cy_2^sCy_2^s$ × *lm lm* $Cy_1Cy_1Cy_2Cy_2$	14	1	*Lm*	$Cy_1 + Cy_2$	122	9	22	0	131	22	153		9,205**	0,035				
		31							2839	882	3721		3,337			32,876		

[1] Except fam. 2005. [2] Fam. 2005.

the low type, *i. e.* through smaller dimensions in all parts of the plant which results in a generally more delicate appearance. The difference in height between tall and microtall is usually very pronounced, but occasionally under-developed tall plants may be mistaken for microtall. Fig. 9 shows the difference between a tall plant and a microtall at an early stage, and Fig. 10 shows two fully developed microtalls.

F_2 results of crosses Nos. 1, 6, 7 and 8 (Table 1) show that the four types appear in the proportions 9 : 3 : 3 : 1. In the cross Chiando × Acacia microdwarf there is a significant excess of *le* plants. The reason for this is not clear. There is no evidence of linkage between *Le* and *Lm* in these crosses.

The F_3 results of cross No. 1, de Winton × Micro, confirm the dihybrid F_2 segregation, but there is a significant deficiency of microdwarfs in low families segregating in *Lm—lm*. The reason for this is as follows.

The whole F_3 generation was sown in the field and no microplants were transplanted to pots or boxes. But while the tall families were classified soon after germination the low families were not examined until later in the summer. Table 3 shows that the percentage of seeds which have given classified plants is much higher in families homozygous for *Lm* than in the segregating families. This indicates that many of the microdwarfs in the segregating families have died before these families were classified. This mortality amongst the microdwarfs is sufficient to explain the deficiency. It is worth noting that in spite of the bad segregation ratio the fifteen segregating families show no sign of heterogeneity. The chi-square calculated with the aid of the BRANDT—SCHNEDECOR formula, is only 7,495 for 14 degrees of freedom.

From the results of F_2 and F_3 it is clear that the micro gene is not allelomorphic to *Le* and that it does not show linkage with the *Le* locus. The microtall has the genotype *Le lm lm*.

This genotype of microtall is confirmed by the result of cross No. 4 between the low Witham Wonder line and a microtall from F_3 of cross No. 1. F_1 consisted of two tall plants and three low plants. The microtall parent had apparently been heterozygous for *Le*. The two tall F_1 plants produced an F_2 generation, the results of which are recorded in Table 1.

Crosses Nos. 5 and 5 a give information about the relation between »micro» and the cryptodwarf type. »Cryptodwarf Lamm» which was used as maternal parent in these crosses originates from a cross »de Haan 204. 1» × »cryptodwarf Nilsson». Its phenotype is that typical for cryptodwarfs and its genotype has been confirmed in other crosses

TABLE 2. *F_3 analysis.*

Cross	Genotype of F_2 parent	Number of families			Genes involved		Number of plants							% classified plants	χ^2 for deviation			χ^2 for heterogeneity		
		Found or estimated	Expected	(a - mn²) / mn							Total		Grand total							
					A	*B*	*AB*	*Ab*	*aB*	*ab*	*Lm*	*lm*			*Aa*	*Bb*	Linkage	*Aa*	*Bb*	Linkage
1. de Winton × Micro	*Lm Lm Le Le*	7	5,11		*Lm*	*Le*	314						314	89,7						
	Lm lm Le Le	11	10,22		*Lm*	*Le*	402		135		402	135	537	97,6	0,006			8,227		
	Lm Lm Le le	9	10,22		*Lm*	*Le*	332	95					427	95,9		1,724			10,162	
	Lm lm Le le	19	20,45		*Lm*	*Le*	505	194	179	51	699	230	929	98,2	0,029	0,933	2,874	16,225	25,523	14,244
	Total	46	46,00																	
	Lm Lm le le	10	8,33		*Lm*	*Le*		232					232	93,5						
	Lm lm le le	15	16,67		*Lm*	*Le*		252		45	252	45	297	81,1	15,363***			7,495		
	Total	25	25,00																	
	lm lm Le Le	2	3,67		*Lm*	*Le*			11				11	64,7						
	lm lm Le le	9	7,33		*Lm*	*Le*			67	26			93	87,7		0,434			—	
	Total	11	11,00																	
3. Acacia × Micro	*Lm Lm*	11	16,67																	
	Lm lm	39	33,33		*Lm*						733	226	959	98,3	1,051			24,666		
	Total	50	50,00																	

3. Aca	Total	50	50,00																		
5 and 5 a. Cryptodwarf Lamm × microdwarf	$Cy_1Cy_1Cy_2Cy_2$	52,548	58,334	0,5739																	
	$Cy_1cy_1Cy_2Cy_2$ / $Cy_1Cy_1Cy_2cy_2$	28,894	33,333	0,5911																	
	$Cy_1cy_1Cy_2cy_2$	43,558	33,333	3,1366																	
	Total	125,000	125,000	4,3016																	
	Cy_1Cy_2 Lm Lm	23	41,67																		
	Cy_1Cy_2 Lm lm	102	83,33		Lm						4217	1313	5530		4,659			104,548			
	Total	125	125,00																		
	$cy_1cy_1cy_2{}^ccy_2{}^c$ Lm Lm	1	2,33																		
	$cy_1cy_1cy_2{}^ccy_2{}^c$ Lm lm	6	4,67		Lm						166	61	227		0,424			9,798			
	Total	7	7,00																		
5. Cryptodwarf Lamm × microdwarf F_2 fam. 2005	$Cy_1Cy_1cy_2{}^ccy_2{}^c$ Lm Lm	0	1,22																		
	$Cy_1cy_1cy_2{}^ccy_2{}^c$ Lm Lm	1	2,44			Cy_1	56	12					68			1,961					
	$Cy_1Cy_1cy_2{}^ccy_2{}^c$ Lm lm	3	2,44		Lm		100		30		100	30	130		0,256			7,937			
	$Cy_1cy_1cy_2{}^ccy_2{}^c$ Lm lm	7	4,90		Lm	Cy_1	207	54	57	15	261	72	333		2,027	3,252	0,027	8,643	0,767	1,980	
	Total	11	11,00																		
	$cy_1cy_1cy_2{}^ccy_2{}^c$ Lm Lm	3																			
	$cy_1cy_1cy_2{}^ccy_2{}^c$ Lm lm	2			Lm						42	9	51		1,471			—			
	Total	204									6872	2121	8993		9,603**			203,222			

TABLE 3. *The gene Lm in dihybrid*

Gene	Cross Nos.	No. of Families	Type of Cross	*Lm* +	*Lm* −	*lm* +	*lm* −	Total	% Classified Plants
Le	1, 6, 7, 8	9	Coupling	765	243	211	81	1300	88,6
»	4	3	Repulsion	186	66	57	31	340	94,4
P	1, 2, 3, 6, 7	24	Coupling	2112	60	48	534	2754	80,5
V	1, 2, 3	15	»	911	296	215	83	1505	80,9
B	1	2	Repulsion	350	94	83	27	554	93,7
St	1	2	»	353	91	97	26	567	95,9
K	1	2	»	344	98	76	18	536	90,6
Wb	1	2	»	339	105	89	28	561	94,9
S	1	2	»	341	100	82	16	539	91,2
A	2, 3, 5	16	»	833	242	246	69	1390	75,2
I	2, 5, 5 a, 6	12	»	837	268	255	90	1450	81,4
R	5, 6, 7	5	Coupling	499	131	138	54	822	91,3
»	2, 3	13	Repulsion	660	97	155	47	959	65,8
Tl	6, 7	2	Coupling[1]	274	66	67	29	436	85,6
»	3	8	Repulsion	464	74	123	42	703	78,9
[1]) Cy_1	5	8	»	259	68	75	24	426	88,1
[2]) Cy_2	5, 5 a	12	»	297	104	90	33	524	91,2
Wa	5, 5 a	8	»	2458	814	812	236	4320	93,5
Wlo	5 (F_3), 8	6	Coupling	269	5	4	83	361	82,2
»	5, 5 a	8	Repulsion	3566	1806	1691	12	7075	90,0
Pl	6, 7, 8	7	Coupling	425	108	74	73	680	77,6

[1] Only F_2 family 2005/41 and corresponding F_3 families.

[2] Only F_3 families showing linkage cy_2—*wa* and showing certain monohybrid segregation Cy_2—cy_2.

not reported here. The pollen parent in cross No. 5 was a microdwarf originating from cross No. 2, Witham Wonder × Micro, and in cross No. 5 a a microdwarf from cross No. 3, Acacia × Micro. F_2 of cross No. 5 consisted of two families in which both *Cy* genes segregated and one family, No. 2005, in which only one of the *Cy* genes, Cy_1, segregated. The microdwarf used as pollen parent in cross No. 5 must therefore have been heterozygous at the Cy_2 locus.

That it was, in fact, the Cy_1 gene which segregated in F_2 family 2005 is proved by the fact that there was no linkage with *Wa*, a gene affecting the wax coating of the plant. *Wa* is closely linked with the Cy_2 locus but is inherited independently of Cy_1 (DE HAAN, 1927, 1930; LAMM, 1947).

Cross No. 5 a gave three F_2 families in all of which both *Cy* genes

segregation with 17 other genes.

χ^2 for deviation			χ^2 for heterog. between crosses				χ^2 for heterog. between fams.			
Lm — *lm*	+ −	Linkage	D. F.	*Lm* — *lm*	+ −	Link-age	D. F.	*Lm*—*lm*	+ −	Linkage
4,468*	0,004	1,489	3	1,204	10,987*	2,984	5	5,327	9,547	1,988
0,141	2,259	3,012					2	0,138	0,267	1,401
21,965***	17,294***	2148,799***	4	9,127	5,068	8,182	19	15,416	19,497	48,168***
21,699***	0,0268	1,412	2	4,854	1,932	0,064	12	11,377	3,679	11,794
7,819**	2,948	0,535					1	0,124	0,087	0,339
3,307	5,762*	0,104					1	0,001	0,448	0,679
15,920***	3,224	0,378					1	0,101	0,382	0,301
5,139*	0,500	0,0160					1	0,130	0,875	0,0001
13,364***	3,479	1,724					1	0,429	0,058	0,606
4,053*	5,112*	0,001	2	4,656	4,824	0,694	13	20,485	15,545	10,887
1,126	0,0745	0,466	3	4,410	0,248	8,096*	8	5,273	8,491	15,377
1,183	2,727	4,283*	2	0,926	0,973	1,038	2	2,443	2,093	4,399
7,925**	50,987***	13,654***	1	0,0198	3,646	1,802	11	19,293	14,382	15,853
2,067	2,398	4,714*	1	0,0062	0,718	0,0997				
0,877	27,084***	12,018***					7	5,510	5,470	9,668
0,704	2,632	0,552					7	10,881	1,621	3,090
0,651	0,366	0,0305	1	0,003	0,537	0,294	10	13,558	10,253	14,215
1,264	1,111	2,253	1	0,252	0,689	0,193	6	5,467	1,512	8,880
0,156	0,0748	301,053***	1	0,205	0,270	0,496	4	4,474	5,861	9,201
3,259	1,828	729,823***	1	0,112	0,088	0,025	6	5,429	8,099	8,429
4,149*	0,949	46,944***	2	0,122	6,232*	4,044	4	4,764	2,759	5,995

segregated. In Tables 1 and 2 the data from cross No. 5 a have been pooled with the data from those two families of cross No. 5 in which both *Cy* genes segregated.

Four types were found in F_2 of crosses 5 and 5 a: low, microdwarf, cryptodwarf and micro-cryptodwarf. The last of the four types can be distinguished from microdwarf immediately after germination as it shows the rapid initial growth which is typical also for the ordinary cryptodwarf. The difference in height between micro-cryptodwarf and microdwarf is maintained throughout development. In the later stages the micro-cryptodwarf might easily be mistaken for a microtall, but usually it can be distinguished from this through a certain variability in the length of the internodes. This peculiarity in the development of the internodes is also found in the cryptodwarfs originating from

LAMM's, RASMUSSON's and NILSSON's material. It thus appears to be an effect typical for the genotype $cy_1\ cy_1\ cy_2^c\ cy_2^c$. The micro-cryptodwarf is easily distinguished from the ordinary cryptodwarf by a difference in size of all parts of the plant.

The F_2 results of crosses 5 and 5 a show that *Lm* is not allelomorphic to Cy_1 or Cy_2 and that it is inherited independently of these two genes.

The data in Table 2 indicate that the *Cy* genes show normal behaviour in F_3 of the crosses 5 and 5 a. The agreement was tested by the method described by BONNIER and TEDIN (1936).

Among the 125 F_3 families only 23 were homozygous for *Lm* whilst 102 showed segregation. The explanation of this poor 1 : 2 ratio is to be found in the way in which the parents were selected. As will be shown later, there is a strong linkage between *Lm* and *Wlo*, a gene which determines presence or absence of wax coating on the upper surface of the leaves. As the recessive, *wlo*, comes from »Cryptodwarf Lamm» and the dominant, *Wlo*, from »Micro», most low *Wlo* plants in F_2 were heterozygous, *Lm lm*, and most low *wlo* plants were homozygous, *Lm Lm*. When mother plants for F_3 were selected, *Wlo* plants and *wlo* plants from the low class in F_2 were taken approximately in the ratio 4 : 1, and as a consequence families heterozygous for *Lm* became overrepresented in F_3.

The seven families originating from cryptodwarf plants in F_2 behaved as expected with regard to the segregation *Lm—lm*.

Unfortunately all microdwarfs and micro-cryptodwarfs in F_2 died before reaching maturity because of unfavourable conditions after transplanting, and it was therefore impossible to obtain any data on the breeding behaviour of these types in F_3. In one F_3 family segregating in *Lm* and Cy_2, but homozygously recessive for cy_1, seed was saved from four microdwarfs. In F_4 two of the plants produced only microdwarfs, 6 and 4, respectively. The other two gave families segregating in microdwarfs and micro-cryptodwarfs. The numbers observed were 5 : 1 and 6 : 3. One microdwarf and one micro-cryptodwarf from the last family were propagated. The offspring of the microdwarf again showed segregation (numbers not recorded) but the micro-cryptodwarf produced in F_5, F_6 and F_7 only micro-cryptodwarfs. These results, although very scant, indicate that the two types behaved as expected.

The data from F_3 families originating from F_2 family 2005 confirm the monohybrid segregation normal : cryptodwarf in that family. They also confirm the independent inheritance between *Lm* and Cy_1.

Data on the relation between microdwarf and slender are available only from cross No. 12, Slender × Micro. Slender originates from DE HAAN's material. F_1 of this cross was of the ordinary low type.

The F_2 data are rather meagre (see Table 1). They show a serious deficiency of *lm lm* plants, which has not been accounted for. The segregation normal : slender is a good fit to 15 : 1, thus confirming that Micro is $Cy_1\ Cy_1\ Cy_2\ Cy_2$.

The expected microslender did not appear in F_2. Eight of the slenders were tested in F_3. Five gave only slenders (plant numbers very small, 4, 4, 12, 4 and 6), but three produced slenders and microslenders. The total of these three families was 20 slenders : 4 microslenders which is a reasonable fit to 3 : 1. Some microdwarfs were also tested in F_3 and one of these gave 13 microdwarfs and 7 microslenders.

The microslender has also been recovered from a cross between Cryptodwarf Lamm (*Lm Lm* $cy_1\ cy_1\ cy_2{}^c\ cy_2{}^c$) and Acacia microdwarf (*lm lm* $Cy_1\ Cy_1\ cy_2{}^s\ cy_2{}^s$). This cross was made for quite a different purpose and was not classified for the cryptodwarf and slender characters. No segregation figures are therefore available but the microslender was observed in several F_4 families.

The microslender differs from the micro-cryptodwarf in the same way as a slender differs from a cryptodwarf, *i. e.* through more lank growth, through longer and thinner internodes and greater height. The approximate height of a microslender is 40—70 cm whilst the slenders reach a height of 1,5—2 metres and more. Fig. 12 shows a young specimen side by side with an ordinary slender.

The results concerning the relation between micro and other height types in peas can be summarized as follows: *Lm* is non-allelomorphic to *Le*, Cy_1 and Cy_2 and so a fourth locus affecting height in *Pisum* has been discovered. In interaction with different allelomorphs at the other three loci, the recessive gene *lm* produces the following phenotypes:

le le Cy_1 and/or Cy_2 *lm lm*	microdwarf
Le Le Cy_1 and/or Cy_2 *lm lm*	microtall
le le $cy_1\ cy_1\ cy_2{}^c\ cy_2{}^c$ *lm lm*	micro-cryptodwarf
le le $cy_1\ cy_1\ cy_2{}^s\ cy_2{}^s$ *lm lm*	microslender

These four types may be described as small scale models of the corresponding *Lm* types. So far no indication has been found that they differ from these in anything but size.

LINKAGE RELATIONS OF THE GENE Lm.

It has been possibe to study the linkage relations between *Lm* and the following 17 genes: *Le*, *B*, *St*, *K*, *S*, *A*, *I*, *R*, *Tl*, *Wlo*, *Pl*, *P*, *V*, Cy_1, Cy_2, *Wa*, *Wb*.

The phenotypical expressions of the first 11 genes are unmistakable and there can be no doubt about their identity. The gene *P* can, however, easily be confused with *V* and on the phenotype alone it is impossible to distinguish Cy_1 from Cy_2 or *Wa* from *Wb*.

The genes *P* and *V* both affect the membrane on the inside of the pod. The phenotypical expressions of the two genes are, as a rule, clearly different (RASMUSSON, 1927; LAMPRECHT, 1938) but difficulties in classification are likely to arise when both genes segregate in one cross. Such has been the case in F_2 of crosses 1, 2 and 3. Results from these F_2 families have been used for the detection of linkage only but not for the calculation or recombination values. For this purpose families have been used where only one of the genes was involved.

Cy_1 and Cy_2 are complementary genes and only doubly recessive plants are phenotypically distinguishable as cryptodwarfs or slenders. Cy_2 is closely linked to *Wa*. With the aid of this linkage it has been possible to separate the two kinds of families in F_3 of the crosses 5 and 5 a provided that they were heterozygous for *Wa*.

The two genes *wa* and *wb*, which have a very similar effect on the wax coating of the plant, have not segregated together in any of the crosses.

The dihybrid ratios are presented in Table 3 together with a χ^2 analysis of the results. Because of the fact that a large proportion of the micro plants gradually died off, we find that the ratio *Lm* : *lm* to a very large extent depends on the other characters for which the plants have been classified. If the other character could be scored on the seeds before sowing (*I* and *R* except in crosses 2 and 3) or on the young plants soon after germination (*Le*, *St*, *Wb*, *Tl*, Cy_1, Cy_2, *Wa*, *Wlo*, and *A*) the ratio *Lm* : *lm* is usually a good fit to 3 : 1. When flower characters (*B*, *K*) are involved, we find more serious deficiencies in the *lm* class and even worse is the result when the pods or seeds had to be classified (*P*, *V*, *S*). An exception is the gene *Pl* (black versus white hilum). Most of the microplants in the crosses involving this gene (crosses 6, 7, and 8) developed pods and seeds and therefore there is only a slight deficiency in the *lm* class. These three crosses were sown in cardboard cartons and the seedlings transplanted to the field. Thus these results demonstrate

the superiority of this method over the method of transplanting the microplants from the field to pots or boxes which was employed in crosses 1, 2 and 3.

Most of the segregation ratios for the other genes are good. Exceptions are *P*, *St*, *A*, *R* and *Tl*. The deficiency in the *pp* class is without doubt due to the linkage between *Lm* and *P*. There is no obvious explanation of the shortage of plants in the *st st* class. This deviation is, however, not very serious and may be due to chance only (P=about 0,02). Nor is the shortage of white flowering plants, *aa*, very serious but in this case the explanation is presumably inferior germination power of seed with the genotype *aa*. The following figures indicate that there is a correlation between percentage of germination and the proportion of white flowering plants:

Cross No.	% germinated seeds	% *aa* plants
2	57,3	18,7
3	81,2	22,1
5	98,7	26,1

The same observation was made by RASMUSSON (1927) who concluded that embryos of the genotype *aa* have a lower vitality than *AA*- and *Aa*-embryos and that this difference in vitality exists also when embryos of the three genotypes have been produced on an *Aa* plant and therefore, because of the pleiotropic effect of *A*, are enclosed in a coloured seed coat. The bad ratio for *R—r* appears to be due to poor germination power of the wrinkled seeds (genotype *rr*). Unfortunately the seeds from those F_1 plants, whose progenies have given the results recorded in Table 3, were not classified into round and wrinkled before sowing. But several other F_1 plants from each of the two crosses concerned have been so classified. In these cases there has been no deficiency of wrinkled seeds. It is well known that wrinkled seeds are inferior to round seeds in germination power (see LAMPRECHT, 1937, p. 293).

Finally, the disturbance of the segregation *Tl—tl* can be explained as a consequence of the linkage *R—Tl*.

The χ^2 analysis reveals slight heterogeneity in the single gene ratios *Le—le* and *Pl—pl*. There is no obvious explanation for these discrepancies, but they cannot be considered serious enough to invalidate the results.

With regard to the χ^2's for linkage the following combinations have given highly significant values: *Lm—P*, *Lm—Wlo* and *Lm—Pl*. There

are also serious disturbances in the dihybrid segregations *Lm—R* and *Lm—Tl*. There is an excess of plants in the classes *lm r* and *lm tl*, irrespective of whether the crosses are in the coupling or repulsion phase. Therefore the deviations from the 9 : 3 : 3 : 1-ratio cannot be caused by linkages *Lm—R* and *Lm—Tl*.

As *R* and *Tl* are strongly linked the two discrepancies presumably have the same cause.

All the crosses and all the families within the crosses agree in having an excess of double recessives, a fact which is reflected in the low heterogeneity chi-squares in Table 3. The discrepancies are, however, most serious in those crosses which have large deficiencies of *rr* plants. These deficiencies are due to poor germination of the *rr* seeds as evidenced by the low percentage of classified plants in these crosses.

Fifteen F_3 families which segregated in *Lm* as well as *Tl* gave together the following dihybrid ratio:

Lm Tl	*Lm tl*	*lm Tl*	*lm tl*	Total	% classified plants	χ^2 for deviation *Lm* : *lm*	χ^2 for deviation *Tl* : *tl*	Linkage
210	69	62	19	360	98,9	1,200	0,0593	0,0444

These families which have a very high proportion of classified plants give excellent 3 : 1-ratios and no indication of linkage.

It is probable that the irregularity of the F_2 segregation has a connection with the poor germination. The data suggest that wrinkled seeds of genotype *lm lm* have kept their germination power better than those of genotype *Lm Lm* or *Lm lm*. Anyhow, the disturbances revealed by the χ^2 analysis are in these cases not due to linkage.

There are in Table 3 two cases of heterogeneity in the linkage data. The first is between crosses involving *Lm* and *I*. This is due to two families in cross No. 5 giving deviations in the »coupling» direction although the cross is of the repulsion type. There is nowhere any indication of linkage between these two genes.

The second case of heterogeneity is between families in crosses segregating in *Lm* and *P*. This discrepancy is highly significant. The contribution of the different crosses to this χ^2 has been analysed with the following results (see next page, above).

This analysis shows that the big heterogeneity χ^2 is largely due to differences between different F_2 families and between different F_3 families in cross No. 3, Acacia × Micro, whereas the total of all F_2 families agrees well with the total of all F_3 families. The discrepancy thus indicates that different families in cross No. 3 differ with regard

Heterogeneity between families in:

	χ^2	D. F.
F_2 of cross No. 1	0,485	1
F_3 » » » 1	0,293	2
F_2 » » » 2	2,253	4
F_2 » » » 3	22,139**	7
F_3 » » » 3	18,397***	3
Total	43,567***	17

Heterogeneity between F_2 and F_3 of:

Cross No. 1	4,519*	1
» » 3	0,082	1
Total	48,168***	19

to the strength of the linkage. But every individual family has given a highly significant linkage χ^2 and, in spite of the heterogeneity, the linkage between *Lm* and *P* is not in doubt. The significance of the linkage χ^2 can be tested through dividing both deviation and heterogeneity χ^2's by their degrees of freedom to give Mean Squares which are then compared by means of Variance Ratio. The deviation Mean Square is in this case 2148,799 and the heterogeneity Mean Square is 2,450 (Table 3). The Variance Ratio is found to be 877,06, a highly significant value.

As *Lm* is linked to *Wlo*, *P* and *Pl*, it is of course to be expected that these genes show linkage with each other. *Wlo* and *Pl* segregate together in cross No. 8, *Wlo* and *P* in cross No. 10, and *P* and *Pl* in crosses Nos. 6 and 7. The dihybrid ratios are found in Table 4.

The single gene ratios are good but there is a certain heterogeneity in the *Pl* : *pl* ratio between crosses 6 and 7. The heterogeneity χ^2 is 7,038 for one degree of freedom which must be considered significant. Nowhere else is there any heterogeneity either between crosses or between families.

NILSSON (1939) found that the genes *Wlo—P—Pl* form a linkage

TABLE 4. *Segregation data for the combinations Wlo—Pl, Wlo—P and P—Pl.*

Genes		Type of cross	Cross No.	Number of families	Number of plants					% classified plants	χ^2 for deviation			χ^2 for heterogeneity		
A	*B*				*AB*	*Ab*	*aB*	*ab*	Total		*Aa*	*Bb*	Linkage	*Aa*	*Bb*	Linkage
Wlo	*Pl*	Coupling	8	5	148	44	29	23	244	66,5	1,770	0,787	8,423**	5,055	1,809	3,650
Wlo	*P*	Repulsion	10	5	170	82	76	0	328	75,6	0,585	0,000	31,306***	2,213	5,125	3,088
P	*Pl*	Coupling	6, 7	2	271	62	51	52	436	85,7	0,440	0,306	40,775***	0,348	7,038**	2,686

group and this is confirmed by the above results. It is clear that this group also includes the gene *Lm*.

RECOMBINATION VALUES FOR THE GENES IN THE LINKAGE GROUP Wlo—Lm—P—Pl.

Lm—P.

The dihybrid ratio for these genes in Table 3 include F_2 families from crosses 1, 2 and 3, where the two genes *P* and *V* segregated simultaneously. As the accuracy of the classification for pod membrane characters is always open to some doubt in such material, it was decided to exclude these F_2 families from the estimation. The recombination value was calculated from three F_3 families of cross 1, one F_2 family of each of crosses 6 and 7 and four F_3 families of cross No. 3. All these 9 families were homozygous for *V* and therefore the classification of the pods into *P* and *p* caused no difficulty.

The nine families gave together:

Lm P	*Lm p*	*lm P*	*lm p*	χ^2 *Lm : lm*	*P : p*
940	25	28	256	3,408	4,170*

Both single gene segregations show a shortage of the recessive but these deviations are hardly large enough to have any serious influence on the recombination values. The maximum likelihood method gives a value of $4{,}49 \pm 0{,}602$ %. The product formula gives $4{,}50$ %.

The data were tested for heterogeneity by the method described by MATHER (1938). The χ^2 analysis gave the following result.

	Heterogeneity χ^2	D. F.
Between crosses	1,577	3
» families		
Within cross 1	0,112	2
» » 3	13,115**	3
Total	14,804	8

This analysis gives the same kind of result as the χ^2 analysis of the dihybrid ratios in Table 3, *i. e.* the different crosses agree well with each other but there is a significant heterogeneity between different families in cross No. 3 with regard to the strength of the linkage. Similar heterogeneity between descendants from different F_2 plants was found by

RASMUSSON (1934) for the genes *Le* and *V*. RASMUSSON considers the most probable explanation to be segregation of crossing over determining genes in F_1, but in this case there is evidence of heterogeneity also between F_2 families and this throws some doubt on the homogeneity of the parent lines, especially on the line Acacia, as there are no disturbances of this kind in other crosses which have »Micro» as one parent.

The exclusion of the F_2 families of crosses 1, 2 and 3 from the estimate has not altered the recombination value very much. The figures in Table 3 which include all available data on the combination *Lm—P*, give an estimate of 4,22 % when the product formula is used. But the value $4{,}49 \pm 0{,}602$ % will be used as the most reliable estimate of the percentage of recombination between *Lm* and *P*.

Lm—Wlo.

The following data are available for calculation of the recombination between *Lm* and *Wlo*:

Coupling phase.

Cross No.	Generation	*Lm Wlo*	*Lm wlo*	*lm Wlo*	*lm wlo*	Total	χ^2 for deviation *Lm* : *lm*	χ^2 for deviation *Wlo* : *wlo*
8	F_2	220	4	3	70	297	0,028	0,001
5	F_3	49	1	1	13	64	0,333	0,333
Total		269	5	4	83	361	0,156	0,075

Repulsion phase.

5	F_2	428	235	227	1	891	0,165	1,051
5	F_3	3138	1571	1464	11	6184	4,348*	1,118
Total		3566	1806	1691	12	7065	3,259	1,829

The single gene ratios are good apart from a slight deviation in the *Lm* : *lm* ratio in F_3 of cross No. 5.

Further 154 of the plants in F_2 of cross No. 5 (repulsion phase) could with the aid of the F_3 results be fully classified as follows:

	Lm Lm	*Lm lm*	
Wlo Wlo	0	3	
Wlo wlo	6	1 [1]	120
wlo wlo	23	1	

[1] double cross-over.

The method of maximum likelihood gives as a combined estimate of all available data the recombination value 5,27 % but the χ^2 test shows

that the material is not uniform. The heterogeneity χ^2 was found to be $18{,}969^{***}$ for 4 D. F. which is highly significant. This χ^2 has been calculated according to the formula $\chi^2 = S\left(\frac{D^2}{I}\right)$ (MATHER, 1938) and D and I have been determined arithmetically from the maximum likelihood expression.

The different groups of data gave individually the following recombination values:

Cross Nr. 8	Coupling F_2	2,39 %
» » 5	» F_3	3,33 »
» » 5	Repulsion F_2, fully classified	3,90 »
» » 5	» », rest	5,87 »
» » 5	» F_3	8,56 »

The coupling phase families and the fully classified part of the repulsion F_2 show fairly good agreement but the rest of the repulsion F_2 and the repulsion F_3 have given considerably higher values, particularly the latter. Now there were over 1500 *Lm wlo* plants in F_3 and it is quite possible that among these several might have been under-developed and therefore erroneously been included in the doubly recessive class. The classification was carried out soon after germination but owing to the great number of plants the supposed dwarfs had to be left in the field and many of them had already died when the material was examined the second time.

Recombination values calculated from repulsion phase data are extremely sensitive to the number in the doubly recessive class, a fact which is reflected in their large standard error. But the standard error indicates the range of variation to be expected due to chance only, and it does not take into account risks of erroneous classification such as we meet in the F_3 data of cross No. 5. In view of this the F_3 repulsion families have been excluded from the estimation. The F_2 repulsion data have, however, been retained as in this case the microdwarfs were transplanted and re-examined and therefore the risk of misclassification considerably reduced.

With the aid of the method described by FISHER (1938, para. 57. 1) a combined estimate of the recombination value was made from families in coupling phase and from fully and incompletely classified plants in repulsion F_2. The value arrived at was $3{,}387 \pm 0{,}689$ %. There is satisfactory agreement between the three groups of data, the χ^2 for heterogeneity calculated in the same way as before, being 2,364, for 2 degrees of freedom.

Lm—Pl.

The recombination value has been calculated from the data given in Table 3, *i. e.* F_2 results from crosses 6, 7 and 8, all in the coupling phase. There is a slight deficiency of *lm* plants. The maximum likelihood formula gives the value 32,75 % and the product formula gives 32,18 %.

The χ^2 analysis gave the following result:

	χ^2	D. F.
Heterogeneity between crosses	2,418	2
» » families	4,897	3
Total	7,315	5

Two of the F_2 families in cross No. 8 had to be pooled as the expectation in the class of the double recessives fell below five. There is no evidence of heterogeneity either between crosses or between families.

The value 32,18 % $\pm$ 2,256 % has been chosen as the best estimate of the recombination fraction for *Lm* and *Pl*.

P—Pl.

Information about the linkage between *P* and *Pl* has been obtained from crosses 6 and 7 both in the coupling phase. The segregation data have been given in Table 4. The single gene ratios are good. The maximum likelihood formula gives a recombination value of 30,69 $\pm$ 2,75 %. The χ^2 for heterogeneity between the two crosses is 1,022, which indicates good agreement.

Linkage between *P* and *Pl* has previously been found by WELLENSIEK (1927), WINGE (1936), NILSSON (1929), and LAMPRECHT (1948). The latter gives the mean crossing over value as 32,7 $\pm$ 1,36. Later NILSSON (1950) found 30,6 % in a back cross.

Wlo—Pl.

These two genes segregated together in cross No. 8. The data in Table 4 give with the maximum likelihood method a recombination value of 37,29 $\pm$ 4,08 %. There is no heterogeneity between the different families of cross No. 8, the χ^2 being 3,116 for 3 degrees of freedom (two of the five families were pooled).

ERNST NILSSON (1939) found 32,1 % and later (1950) 36,9 %.

P—Wlo.

The only data on the linkage *P—Wlo* in this investigation are provided by cross No. 10 (Table 4).

Owing to the absence of plants in the doubly recessive class these results give a value of 0 as the best estimate of the recombination value. No F_3 data are available.

Linkage between *P* and *Wlo* has previously been found by NILSSON (1939) and LAMPRECHT (1939). They obtained the following results:

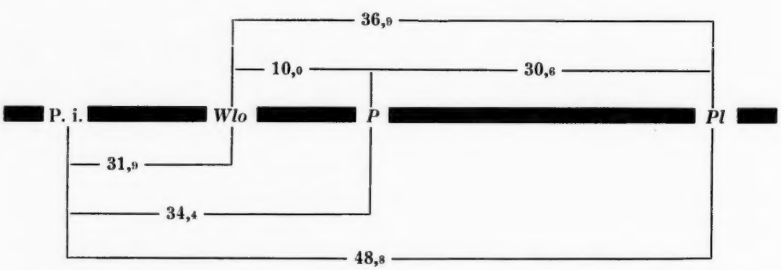
	Wlo P	*Wlo p*	*wlo P*	*wlo p*	Total	χ^2 for 3 : 1 *Wlo* : *wlo*	χ^2 for 3 : 1 *P* : *p*
NILSSON (coupling)	1421	110	67	379	1977	6,280	0,074
» (repulsion)	639	340	283	1	1263	4,257	2,692
LAMPRECHT (repulsion)	239	107	111	2	459	0,036	0,384

If NILSSON's, LAMPRECHT's and my own data are combined the maximum likelihood method gives a recombination fraction of $9{,}459 \pm \pm 0{,}674$ %. The χ^2 for heterogeneity is 3,749 for 3 D. F. which indicates good agreement between the crosses. NILSSON (1950) found 10,0 %.

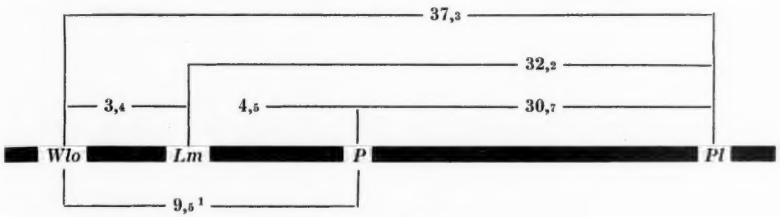
The results concerning the recombination values for the genes in the linkage group *Wlo—Lm—P—Pl* can be summarized as follows:

Gene combination	Recombination value
Wlo—Lm	$3{,}39 \pm 0{,}69$ %
Wlo—P	$9{,}46 \pm 0{,}67$ »
Wlo—Pl	$37{,}29 \pm 4{,}08$ »
Lm—P	$4{,}49 \pm 0{,}60$ »
Lm—Pl	$32{,}18 \pm 2{,}26$ »
P—Pl	$30{,}69 \pm 2{,}75$ »

NILSSON (1950) found in a backcross generation comprising 160 plants the following linear relation between the genes *Wlo*, *P* and *Pl* and the point of interchange of the N.III translocation:



This four point cross leaves no doubt about the arrangement of the genes *Wlo*, *P* and *Pl* on the chromosome. My data suggest the same order with *Lm* located between *Wlo* and *P*.



[1] NILSSON's and LAMPRECHT's data included.

The recombination values for *Wlo—Pl* and *P—Pl* from NILSSON's backcross agree extremely well with those found in my crosses. If, however, other published segregation data are taken into account less satisfactory agreement is found. As has already been mentioned, LAMPRECHT found a mean crossing over value of $32{,}7 \pm 1{,}36$ for *P* and *Pl*. Combined estimation from NILSSON's data (NILSSON, 1939) and my own give for *Wlo* and *Pl* a recombination fraction of $32{,}8 \pm 1{,}17$ %. These combined values leave open the question of the order of the genes *Wlo—P—Pl*. But this matter must be considered as settled by NILSSON's four point back cross data which are supported by the results presented here.

The recombination value for *Wlo—P* is significantly larger than either the value for *Wlo—Lm* or the value for *Lm—P*, which proves that *Lm* is situated between *Wlo* and *P*. The total of the values for *Wlo—Lm* and *Lm—P* is in fact smaller than the value for *Wlo—P* although not significantly smaller.

The good agreement between different crosses with regard to recombination values for the genes in this linkage group deserves to be emphasized. The following heterogeneity χ^2's have been found for the different gene combinations:

Genes	Heterogeneity between Crosses		Heterogeneity between Families	
	χ^2	D. F.	χ^2	D. F.
Lm—P	1,577	3	13,227*	5
Lm—Wlo	2,364	2		
Lm—Pl	2,418	2	4,897	3
P—Pl	7,193	7		
Wlo—Pl	1,542	1		
P—Wlo	3,749	3		

Results published by the following authors have been included in these calculations:

On *P—Pl*, WELLENSIEK (1927), NILSSON (1929), WINGE (1936).
On *Wlo—Pl*, NILSSON (1939).
On *P—Wlo*, NILSSON (1939), LAMPRECHT (1939).

The heterogeneity between families with regard to strength of the linkage *Lm* and *P* has been discussed above. It is the only indication found here of genetically determined variation in linkage values, so often encountered in *Pisum*.

DISCUSSION.

The fact that a new gene for height, *Lm*, has been located close to the gene *P*, affecting the membrane on the inside of the pod, is of interest in connection with the demonstration of duplications in *Pisum*. The gene *P* is in its phenotypical manifestation similar to the other pod membrane gene, *V* (RASMUSSON, 1927; LAMPRECHT, 1938), and several authors have expressed the opinion that *P* and *V* might well be duplicate genes (LAMM, 1947; LAMPRECHT, 1949). It has long been known that *V* is linked to *Le*, the major gene for height (RASMUSSON, 1927; LAMPRECHT, 1948). When it is now found that *P* is also linked to a gene affecting height it is very tempting to assume that the two chromosome segments carrying *Le—V* and *Lm—P* have originated through duplication of one ancestral segment.

LAMPRECHT (1949) concluded that *P* and *V* are duplicate genes, partly from their similarity in phenotypical expression and partly from the fact that the recessive allelomorphs of both genes have a tendency to mutate to the dominant and that these mutations at both loci are often associated with structural changes in the chromosomes. LAMPRECHT goes so far as to prophesy, without knowledge of the linkage *Lm—P*, the existence of a height gene in the neighbourhood of *P*.

The genes *Le*, *V* and *P* were by RASMUSSON (1927) placed on the same chromosome, but his results have not been confirmed. The situation is by no means clear, but in view of later evidence it is now fairly certain that the chromosome segments carrying *Le—V* and *Lm—P* are located on different chromosomes. NILSSON (1950) has recently discussed this question.

The genes *Lm* and *P* gave a recombination value of $4{,}49 \pm 0{,}602$ %. LAMPRECHT (1948) gives recombination values for *Le* and *V* from 50 different crosses by different workers. The values vary from 2,6 % to 38,5 % giving a mean of $12{,}6 \pm 0{,}47$ %. The two pairs of genes, therefore,

do not show good agreement with regard to recombination value. But the strong variation in the linkage between *Le* and *V* is, according to LAMPRECHT (1948), evidence that these two genes are located near the end of a chromosome. In the neighbourhood of *P* he found little variation in crossing over values, a fact which has been confirmed above. This LAMPRECHT interprets as evidence that *P* is situated near the centromere. Now it is known from comparison between genetical chromosome maps and salivary gland maps that the crossing over per cytological unit is greater near the end of the chromosome than near the centromere. It is therefore not impossible that the cytological distances *Lm—P* and *Le—V* are the same in spite of the poor agreement between the genetical recombination values.

The pod membrane genes *P* and *V* are clearly different in their phenotypical expression (RASMUSSON, 1927; LAMPRECHT, 1938). *P* produces patches of weak sclerenchymatic membrane over the whole inside of the pod wall but *V* produces a strip of strong membrane along the ventral seam only. Together they produce a strong membrane over the whole of the inside of the pod wall.

Lm and *Le* are even more different in their phenotypical effects. *Lm* controls the size of all parts of the plant, the recessive causing a drastic reduction, whereas the effect of *Le* is confined to the length of the internodes.

However, absolute similarity can not be expected between two originally identical genes located on the different chromosome segments involved in a duplication. Many leading geneticists hold the view that such genes must be expected to diverge from each other in the course of evolution. Thus MULLER (1940, 1947), HUXLEY (1944), METZ (1947), DOBZHANSKY (1937), and WHITE (1942, 1945) are all of the opinion that duplication of chromosome parts together with divergent specialization of homologous genes is the most important method of gene increase in evolution. STEPHENS and CASSIDY (1947) have come as near as is possible to experimental evidence on this problem in their investigations concerning pseudo-allelism in Asiatic cotton. They studied on the biochemical level the effect of two closely linked genes controlling anthocyanin pattern and found that they control similar chemical reactions (reduction) on different, but chemically closely related substances (anthoxantin and an anthocyanin leuco precursor). Their interpretation is that these genes have originated through duplication followed by divergent specialization.

It is, of course, also conceivable that any difference in the effect of

two duplicate genes is due to position effect. LAMPRECHT (1949) has advanced this explanation for the difference in phenotypical manifestation between *P* and *V*. These two genes appear, however, to have a very specialized interaction, the result of which is a strong sclerenchymatic membrane in the pod. It is not easy to understand how such a mutual adjustment of the action of two genes can be due exclusively to the accidental location of the genes in different chromosomes or chromosome parts. But it is not difficult to imagine that it has been brought about by a gradual divergence of the genes, through mutation or through the building up of systems of modifiers, under the guidance of natural selection.

The discovery of a height gene in close proximity to *P* must be considered as having strengthened the evidence for the existence of a duplication. But in order to settle this question the genetical evidence ought to be augmented by biochemical evidence through studies of the same nature as the investigations by STEPHENS and CASSIDY referred to above. If it could be proved that the height genes *Le* and *Lm* control similar chemical processes and also the pod membrane genes *P* and *V* there could remain little doubt that we are dealing with two sets of originally duplicate genes.

SUMMARY.

The micro-character is conditioned by a single recessive gene, *lm*, which is non-allelomorphic to *Le*, Cy_1 and Cy_2 and which in interaction with these genes produces the phenotypes microdwarf, microtall, micro-cryptodwarf and microslender.

The gene *Lm* is closely linked to *P* in the *Wlo—P—Pl* group.

The possibility that the two chromosome segments carrying the genes *Lm—P* and *Le—V* have originated through duplication of one ancestral segment is discussed.

I am indebted to Svenska Sockerfabriks A.B. for providing the necessary facilities for this investigation. The work was started on the initiative of Professor J. RASMUSSON, Hilleshög, Landskrona. I am very much obliged to him and to Dr. R. LAMM, Alnarp, for material and for helpful advice. Further I owe a great debt of gratitude to Professor K. MATHER, Birmingham, for most valuable guidance. My thanks are

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NEUTRON AND X-RAY EXPERIMENTS IN BARLEY

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INTRODUCTION.

IN a series of papers (1941 a and b, 1942, 1947 a) GUSTAFSSON has given several proofs of the fact that X-ray induced mutations in barley may be progressive, *i. e.* of definite value from agronomic or evolutionary standpoints. In addition to his results practically valuable mutations have been found in X-rayed material of barley (FREISLEBEN and LEIN, 1942, 1944), of wheat (SABNIS and METHA, 1942; GUSTAFSSON and MAC KEY, 1948 a; OLTMANN, 1950), of oats (GUSTAFSSON and MAC KEY, 1948 a), of flax (GRANHALL, 1946; *cf.* GUSTAFSSON and MAC KEY, 1948 a), of soybeans (ANDERSSON and OLSSON, 1947) and, if probably not entirely due to the X-ray effect, of white mustard (ANDERSSON, 1950). These promising results in agricultural plants have stimulated an extension of the Svalöf work on induced mutations. The present paper is with the exception of a very preliminary one (GUSTAFSSON and MAC KEY, 1948 b) the first report on a comparison between the mutagenic effect of X-rays and fast neutrons when treating dry and pre-soaked seed of barley. It presents the data obtained from the first generation and gives a comparison of the ability of the two types of radiation to produce chlorophyll mutations. A more thorough analysis of the second generation will be given in a future paper.

The reasons why neutrons and nitrogen mustard (not to be discussed in this paper) have been introduced in the Svalöf experiments were that these mutagens are known to produce relatively higher proportions of gross chromosome re-arrangements and »point» mutations, respectively, than X-rays produce. It was supposed that a better understanding of the practical value of these two different types of mutations would be obtained by introducing these new agents.

The absence of electrical charge gives both fast neutrons and hard X-rays a high penetrating ability in tissue. Fast neutrons dissipate their energy in biological material mainly by the projection of protons, while X-rays eject electrons. Physically, primary ionization and excitation,

produced by a proton or electron of the same speed, are practically indinstinguishable. This justifies the treatment of these two particles as nearly equivalent from the radiobiological standpoint (GRAY, 1950). Their different biological effect lies in the number and distribution in space of the ionizations and excitations which they produce. According to GRAY (1946, 1950), the radiation sources used in the present experiments should cause a mean linear ion density in tissue of about 380 ions per micron for neutrons and about 100 for X-rays. When comparing these figures, it should be observed that the protons are ejected in approximately straight lines with a rather even distribution of ions along their paths. The electron tracks are, however, definitely shorter, tortuous, and with the ions produced more in clusters, giving the particles a varying efficiency along their ways through the tissue. This difference in ion density, characteristic of neutrons and electrons, causes them to be differently effective from a biological standpoint, if the number of ionizations, necessary to produce the events, varies. Thus X-rays must be more effective, when only a single, or a very few ionizations, are needed, while neutrons should be more advantageous, when a higher energy dissipation proves necessary to affect the target (*cf.* GRAY, MOTTRAM, READ, and SPEAR, 1940; FANO, 1944; LEA, 1946). The detailed biological verifications of this statement and its cytological and genetical consequences will be discussed later, together with the data obtained in the present experiments.

The neutron treatments have been carried out at the Nobel Institute for Physics, Stockholm (Director: Prof. Dr. M. SIEGBAHN). I am especially indebted to Dr. A. HEDGRAN and Mr. S. JOHANSSON of this institute for their indispensable help and suggestions, as well as to Dr. A. FORSSBERG at the Radiophysics Institute, Stockholm, for his kind co-operation with regard to the X-ray treatments. Financial support was given by the Swedish Research Council of Natural Science, the Knut and Alice Wallenberg Foundation, and the Nilsson-Ehle Foundation.

MATERIAL AND METHODS.

The present results are based on three series of experiments, initiated in 1947, 1948, and 1949, respectively. The same barley variety has been used all three years, *viz.* a several times reselected and carefully purified, homozygotized line out of the two-rowed Bonus barley, which is characterized by a very high productive capacity. Only seeds have been irradiated, as this mode of treatment is the most reproducible and that

best fitted for practical use in plant breeding. In accordance with the findings of FRÖIER and GUSTAFSSON (1944) on the correlation between seed size and X-ray susceptibility only a certain sieve fraction (2,50—2,75 mm) has been used. The seed has each year been taken from the freshest harvest and stored some time before treatment in room temperature in order to obtain a low, uniform water content, which seems necessary for a good reproductivity of the experiments (GUSTAFSSON, 1947 a). The water content of the dry dormant seeds immediately before treatment was 11,7, 10,4, and 10,4 per cent, respectively, for the three years.

The treated seeds were sown in the field and a certain number of the surviving plants of this first generation (called X_1 and N_1) were analysed as to their proportion of fertile and sterile flowers. All spikes of a plant were separately examined in this way. Thus the correlation between the type of mutation and the type of chromosome disturbance in the first generation as indicated by the seed setting could be based on both the plant and the spike as unit. A maximum of five spikes per plant were examined in X_2 and N_2. One of the spikes was sown in the field, the others in the greenhouse. The field material was analysed for all types of mutations. The greenhouse material was only analysed for chlorophyll mutations and examined winter-time under as constant conditions as possible (temperature: $+ 15 \pm 5°$ C, 4 hours' artificial light of ca. 1500 lux added every night). The frequency of chlorophyll mutations presented in this paper is based on the whole X_2 and N_2 material sown, *i. e.* on both field and greenhouse materials, and the mutations were classified at seedling stage according to the system of GUSTAFSSON (1940). As the intensity of the colour and its distribution over the leaf are to a certain extent dependent on light and temperature, all outdoor mutations were reclassified by sowing X_3 and N_3 material from the mutated lines under greenhouse conditions the following year.

The methods of irradiation have briefly been reported by GUSTAFSSON and MAC KEY (1948 b). As the neutron doses were calculated about sixteen times too high in their paper and the technique was not quite satisfactorily elaborated, one may be justified in recapitulating the dosimetry used.

Neutron treatment. — As neutron source the 80 cm cyclotron of the Nobel Institute for Physics, Stockholm, was used, an equipment described in detail by ATTERLING (1945). The neutrons were produced by bombarding a beryllium target with deuterons accelerated to 6,5—7 MeV. In order to ensure a stable run the neutron beam from the target

was regulated to give an ionization current of $0{,}6 \cdot 10^{-7}$ A in a boron chamber. The fast neutrons produced were inhomogeneous with energies up to approximately 8,5 MeV. The seeds were filled in glass tubes of an outside diameter of 22 mm and a thickness of 1,5 mm. The glass tubes were circularly arranged in a fixed wooden plate between the magnet poles but outside the tank and at a distance of 20 cm from the target on a place, where the intensity of irradiation has proved to be most uniform. The vertical variation in neutron intensity from the centre to the ends of the ten cm high seed column in the glass tubes has been estimated to ca. 10 per cent. In order to exclude the main part of the γ-radiation from the cyclotron, the glass tubes were covered with a Pb plate 2 mm in thickness. The time of exposure ranged from 10 minutes for the lowest dose applied to 90 minutes for the highest one. As the biological action of neutrons is mainly produced by single hits and the metabolic activity of the irradiated dormant seeds is extremely low, the influence of the time factor may not be too important.

Owing to the inherent difficulties in obtaining a constant beam from a cyclotron and the high dosages necessary in the present experiments, a dose integration method by the activation of an indicator has been preferred to direct measurements by means of an Aerion condenser chamber or a Victoreen r-dosimeter, even if these methods should be acceptable for neutrons (*cf.* ZIMMER, 1938, 1941; AEBERSOLD and LAWRENCE, 1942; GRAY, 1944; AEBERSOLD and ANSLOW, 1946). The idea of applying a radioactive reaction as an indication of neutron dose has earlier been proposed by LAWRENCE, AEBERSOLD and LAWRENCE (1936) using the reaction $S^{32}(n, p)P^{32}$ (14,5 days), ZIMMER (1938) using Ag^{108} $(n, 2n)Ag^{107}$ (2,4 min.), GRAY, MOTTRAM, READ, and SPEAR (1940) using $P^{31}(n,p)Si^{31}$ (2,67 hours), and CONGER and GILES (1950) using $In^{115}(n,\gamma)$ In^{116} (54 min.).

With the range of doses and technique of irradiation applied in the present experiments the reaction $Al^{27}(n, \alpha)Na^{24}$ (14,9 hours) was found to be the most convenient one. A ring (inside diameter: 23 mm; thickness: 1 mm; height: 10 mm) of pure Al was placed around the middle of each glass tube during irradiation and after 6—7 hours the intensity of the Na^{24} disintegration ($Na^{24} \rightarrow Mg^{24} + e^- + 2\gamma$) was determined by an arrangement for β—γ-coincidence measurements (HEDGRAN, 1948). As the diameter of the glass tubes and of the Al rings was conformed to that of the GM tube, these measurements could be made with high accuracy. Thanks to the favourable disintegration scheme of Na^{24} the number of disintegrated atoms per gram Al and minute could be calcu-

lated. This number, given for the middle of the exposure time, was then used as a measure of the dose applied. This new unit, disintegrations per gram Al and minute (abbreviated to *dis*), gives a good reproductivity for neutrons of about the same energy as those used here, and does not depend on any special apparatus of measurement.

The α-, β- and γ-radiation produced from the Al ring, as well as any secondary radiation caused by the bombardment of the glass wall of the tube, are negligible from a biological point of view compared with the high primary effects of the neutrons.

In order to allow for a comparison with other experiments on neutrons, where the *n*-unit (*cf.* AEBERSOLD and LAWRENCE, 1942) and the »energy unit» or *r*-unit (*cf.* GRAY, MOTTRAM, READ, and SPEAR, 1940) have been used, a comparing measurement has been performed with a Victoreen r-dosimeter. This has been kindly made by Mr. L. E. LARSSON of the Radiophysics Institute and Mr. S. JOHANSSON of the Nobel Institute for Physics. They found 1 *n* to be equal to 42 *dis*, and thus, if AEBERSOLD's correction factor of 2,5 is accepted, 1 *r* approximately equal to 17 *dis*. It must be pointed out that this comparing measurement is not definite and thus not completely free from objections.

As is clear from the preliminary paper of GUSTAFSSON and MAC KEY (1948 b), the dosage in the experiment of 1947 was determined by a less elaborated method than this one described above. An Al plate was put inside the glass tube with the seed, and the activity was then only measured as the number of impulses given to a small β-tube. As this method was dependent on the individual β-tube used, the following year it was discarded. Though introducing a certain source of error, the doses given in 1947 have been recalculated to *dis*-units in order to make comparisons possible with the experiments of 1948 and 1949.

X-ray treatment. — The X-ray irradiation was performed at the Radiophysics Institute, Stockholm. Lots of 200 grains were laid in a small, round, and flat paper box with lid and bottom 1 mm thick and with the seed arranged in two layers. The X-rays were produced by a Coolidge type X-ray tube (Philip's Metalix) and the applied intensity was measured inside the box on its fixed place before and after each irradiation. In order to obtain as uniform an effect as possible the box was turned around after exactly half the exposure time. The beam was always filtered through 1 mm Al. The tube was operating under the following conditions:

Year	mA	kV	Intensity, r/min.
1947	4	165	780
1948	6	170	1620
1949	6	170	1500

The pre-soaked seed irradiated in 1949 was placed 24 hours before irradiation on a wet filter paper bed with the water covering half to one third of the grain. Immediately before irradiation the seed was wiped off between filter papers.

In all experiments the treated seed was sown in the field the day after irradiation.

EXPERIMENTAL RESULTS.

THE RESPONSE OF THE FIRST GENERATION TO IRRADIATION.

Before entering on a discussion of the experimental results obtained, it may perhaps be convenient to express some views about the chosen research material, the barley seed. From evolutionary aspects the barley species is an ideal object, as all mutations arisen spontaneously or artificially can be appropriately tested in what may be called their »natural habitat», *i. e.* in this case the arable land (GUSTAFSSON and MAC KEY, 1948 b). The agricultural, and thus the evolutionary, value of a mutant can be measured by exact methods, considering both the pure agronomic values (GUSTAFSSON, 1947 a) and the competitive ability determined on generative and vegetative characters (GUSTAFSSON, 1946 a, 1947 b; GUSTAFSSON and NYBOM, 1950). From these points of view the present experiments have also been planned. The highest number of preservable mutations is desired under such circumstances, and this will explain why the seed was preferred to the pollen grain as an object of irradiation and why the doses were chosen as high as around the mean lethal dose (37 per cent survival) of X-rays, a dose range which has been found by GUSTAFSSON (1944) to be the most advisable for practical purpose.

On the other hand it must be pointed out that the barley seed is less suitable for studies on the direct physical and chemical effect of irradiation on living cells. Some interesting information has, however, been obtained together with verifications on the general validity of phenomena earlier observed for vira, bacteria, *Drosophila* sperm, *Tradescantia* pollen or fast-growing plant and animal tissues. Physiological problems, for instance, can be studied with great advantage on such subjects, where the metabolism can be drastically changed by simple means.

From the target-theory point of view it is important to establish that the barley seed is a multicellular object. Further, the size of the »sensitive part» of the seed is not the same, if the death or the induction of a heritable mutation be recorded as the biological event. In the last case one of the 3—4 ear primordia (STADLER, 1928 a) must be hit; and in the first case an affection of the radicula may also cause the registered phenomenon. Each primordium is certainly already in the dormant seed represented by a group of germ line cells and not only by one such single cell. This assumption is based on the pronounced chimaera character of the X_1 and N_1 spikes, often cytologically observed by examination of meiosis in different parts of one spike (in wheat also observed by SMITH, 1946), and on radiogenetical studies on wheat (unpublished data). A probably rather pronounced ability of different cells substituting each other increases the complexity of all target interpretations.

Cytological effects. — The frequency of chromosome disturbances induced in dormant seeds of barley by different X-ray and neutron dosages has been examined by Mr. N. NYBOM in the first mitotic cycle after the germination (rootlets fixed when 8—12 mm long). His unpublished data have kindly been put at the author's disposal. The frequency of cells with fragments gives a linear relationship to the dose with a slope down at higher dosages due to a saturation effect. NYBOM has calculated the relative efficiency of the two radiations in producing this special cytological effect to $dis/r = 0{,}6$. In accordance with results obtained by GILES (1940, 1943), and THODAY (1942) on the induced frequency of interchanges in irradiated *Tradescantia* chromosomes, the bridges produced by neutrons followed a linear relationship to dosage instead of the exponential relation found for X-rays. This is explicable on the assumption that both the exchange breaks are produced by the same ionizing particle after neutron irradiation and thus forms a very important proof of the validity of the target theory. The *dis*/*r* ratio for bridges in the present examination could be calculated to 0,6—0,7, and for the total number of disturbed cells NYBOM found the *dis*/*r* ratio to be about 0,6.

These results can only be compared with other experiments from the estimation of 1 energy unit being equal to 17 *dis*-units. From this information the N/X ratio in the present investigation should be about 10 for the cytological effects studied. This figure seems to be rather high but quite reasonable. Thus MARSHAK (1939) found, if his figures be corrected with AEBERSOLD's factor of 2,5, that the N/X ratio was 2,6, 2,5, 2,6, and 2,3, if the frequency of abnormal anaphases in irradiated

Fig. 1. Decreased sprouting ability of barley seeds irradiated with successively increasing doses of neutron. Nos. 1—6 cover the dose range 1.000—11.000 *dis*. No. 7 is an untreated control.

roots of horsebean, pea and tomato seedlings and mouse tumours, respectively, were considered. The above N/X ratio may also be compared with that found for induction of chromatid dicentrics, iso-chromosome breaks, chromosome breaks and interchanges in *Tradescantia* microspores, where the ratios 5 (GILES, 1940, 1943), $2{,}9$, $3{,}6$, and $4{,}2$ (THODAY, 1942; his values recalculated according to $1\ r = 2{,}5\ n$ instead of $2{,}0\ n$) have been found, respectively. For induction of dominant lethals in *Drosophila* DEMPSTER (1941) gave the N/X ratio $1{,}5$ and FANO (1943) a value approaching 2.

That the N/X ratio obtained by NYBOM is higher than all other cited values may be explained by the fact that dry dormant cells have been irradiated in his case, while hydrated cells with high metabolic activity were used in the other experiments. The observation of MARSHAK (1942) that neutrons have their highest relative efficiency at the resting stage of the nuclear division at least stimulates to such an interpretation of the observed differences.

Effects on the sprouting ability. — Seed irradiated when dry always germinate within the dose range commonly applied in mutation experiments. According to COLLINS and MAXWELL (1936) 2.000.000 *r* were necessary to inhibit germination of all the seeds of maize, while 60.000—100.000 *r* were able to cause total death at seedling stage. Within a more moderate dose range the retardation of growth is the first effect of irradiation observed, in seed experiments nicely illustrated by the influence on the sprouting ability (Fig. 1). If previous data (WERTZ, 1940;

FRÖIER, 1941; FRÖIER and GUSTAFSSON, 1941; FREISLEBEN and LEIN, 1943 a) on the retarding effect of X-rays on sprouting as measured by the length of seedlings be plotted against dose, all experiments give

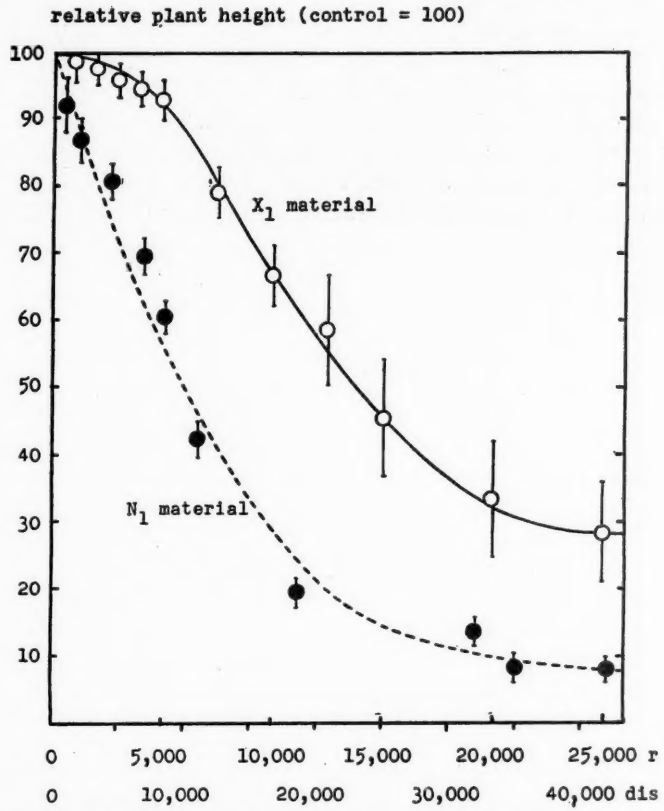

Fig. 2. Diagram showing the relationship in X_1 (unbroken line) and N_1 (broken line) between decreased sprouting ability and dose. Each value is based on 100 seeds of barley sown in sand and the plant height was measured 14 days after sowing. Vertical lines give the standard error 2,5 times. Inter-radiational dose scale: 1 *dis* = 0,6 *r*.

typical sigmoid relations as in the present experiment. After neutron treatments, however, this relationship has been found to be exponential (Fig. 2). If the target theoretical interpretations of the X-ray effect put forward for this special phenomenon by WERTZ (1940) be accepted, the behaviour of the neutrons is not quite unexpected, as the induced chro-

mosome disturbances here seem more to be due to single-unit actions than if X-rays were applied. If the phenomenon should be explained in accordance with DALE's (1940, 1943) physiological inactivation hypothesis, the same difference in effect between X-rays and neutrons could be expected, due to the different spatial ion distribution characteristic of the two radiations. Both explanations must without doubt be accepted. That point events really exist, has *i. a.* been proved by the above discussion on induced chromosome re-arrangements. They must to a certain extent be responsible for the delaying process of regeneration, due to a substitution of killed or weakened cells of the meristems. The existence of growth-factor destruction or transport inhibition and production of diffusible, growth-retarding substances must, however, be considered as well. That these phenomena are of great importance not only when irradiating tissue cultures *in vitro* (*cf.* KING, 1949) but also when treating seeds or seedlings, is shown by JOHNSON (1926), CATTELL (1931), SKOOG (1935), SINGH (1941), and SMITH and KERSTEN (1942). The influence of irradiation on the nucleic acid metabolism (*cf.* DARLINGTON and LA COUR, 1945) may, however, be of less importance in the present case, as all cells were in their dormant stage at the time of treatment.

It may be pointed out that GRAY, MOTTRAM, READ, and SPEAR (1940) have found similar relationships for X-rays and neutrons in inhibiting mitosis in tissue cultures of chick embryo fibroblasts.

Effects on the surviving ability. — The killing effect is often used as a criterion in radiobiology. This has also been the case in studies of the relative radiobiological efficiency of fast neutrons and X-rays. All experiments performed on growing plant and animal tissue have proved neutrons to cause lower survival at comparable doses than X-rays (ZIRKLE and AEBERSOLD, 1936; ZIRKLE, AEBERSOLD, and DEMPSTER, 1937; ZIRKLE and LAMPE, 1938; SPEAR, GRAY and READ, 1938; SPEAR and TANSLEY, 1944; GRAY and READ, 1939, 1943, 1948; GRAY, MOTTRAM, READ, and SPEAR, 1940; GRAY, READ, and POYNTER, 1943; MITCHELL, 1947). In contrast to all these results with fast-growing tissue the surviving ability after neutron treatment of dormant seeds was found to be very high (*cf.* Table 1). When comparing with X-rays it may be observed that the year 1947, due to very unfavourable weather conditions, shows an abnormal high sensitivity to neutrons, as the weak seedlings died to an unexpected extent. Under more normal conditions the resistance has been shown to be so high that an exact information on the mean lethal dose has not been obtainable, although the average plant fertility in the highest doses is considerably below that of the X_1 material. From indoor

TABLE 1. *The X_1 and N_1 effects after irradiation of barley seeds.*

Year	Treatment	No. of grains sown	No. of seedlings, %	No. of mature plants, %	Mean plant fertility, %	Mean No. of spikes per plant
1947	Control, dry seed	300	93,3	83,7	88,9 ± 0,41	4,8 ± 0,27
1948	» , » »	600	94,0	91,8	97,0 ± 0,10	4,1 ± 0,09
1949	» , » »	750	94,9	91,6	96,1 ± 0,13	4,2 ± 0,11
1947	5.000 *r*, » »	300	95,7	80,3	72,9 ± 1,08	3,5 ± 0,18
»	10.000 », » »	300	70,0	52,3	55,2 ± 1,64	4,9 ± 0,35
»	15.000 », » »	300	27,0	21,7	46,8 ± 2,76	9,5 ± 1,17
»	20.000 », » »	300	18,7	17,7	41,0 ± 3,42	10,2 ± 1,44
»	25.000 », » »	300	16,0	11,0	40,0 ± 3,36	13,2 ± 1,63
1948	10.000 », » »	450	92,0	80,9	74,6 ± 1,29	3,7 ± 0,14
»	15.000 », » »	600	81,3	60,3	70,6 ± 1,35	4,1 ± 0,20
»	20.000 », » »	600	74,3	43,5	66,6 ± 1,32	5,0 ± 0,27
1949	5.000 », » »	300	95,0	91,3	90,8 ± 0,81	3,3 ± 0,14
»	10.000 », » »	450	95,6	90,4	75,6 ± 1,81	3,4 ± 0,18
»	15.000 », » »	600	83,0	40,8	77,0 ± 2,06	4,1 ± 0,22
»	20.000 », » »	750	57,6	26,7	77,3 ± 1,94	6,5 ± 0,30
»	5.000 », soaked seed	450	52,2	30,2	80,4 ± 1,71	6,7 ± 0,33
»	10.000 », » »	600	35,3	22,0	71,1 ± 2,19	8,2 ± 0,38
»	15.000 », » »	750	30,4	18,5	71,3 ± 1,79	8,0 ± 0,41
»	20.000 », » »	900	19,7	11,3	72,6 ± 2,15	11,4 ± 0,63
1947	2.500 *dis*, dry series	600	92,0	72,7	66,4 ± 1,10	4,4 ± 0,25
»	4,500 » , » »	600	91,3	74,8	52,7 ± 1,23	4,6 ± 0,25
»	7.000 » , » »	600	90,2	69,0	38,9 ± 1,21	4,8 ± 0,29
»	9.000 » , » »	600	88,5	65,0	27,9 ± 1,10	6,1 ± 0,35
»	13.500 » , » »	600	64,7	30,5	15,6 ± 1,27	5,3 ± 0,38
1948	1.000 » , » »	300	95,7	91,3	86,2 ± 0,66	3,6 ± 0,13
»	1.300 » , » »	300	93,3	89,0	80,1 ± 0,81	3,6 ± 0,13
»	3.300 » , » »	600	91,3	81,5	54,5 ± 1,18	3,6 ± 0,12
»	5.700 » , » »	450	95,3	84,7	54,6 ± 1,12	3,1 ± 0,10
»	6.100 » , » »	600	93,8	85,5	47,0 ± 1,24	4,0 ± 0,15
1949	1.500 » , » »	300	95,3	94,3	85,4 ± 0,94	4,2 ± 0,20
»	4.000 » , » »	300	94,0	93,7	68,4 ± 1,38	3,7 ± 0,15
»	6.400 » , » »	300	94,7	89,7	56,2 ± 1,84	3,0 ± 0,11
»	10.400 » , » »	450	92,7	89,8	37,8 ± 1,54	3,4 ± 0,14
»	14.200 » , » »	600	93,3	79,8	31,1 ± 1,48	3,5 ± 0,16
»	1.400 » , soaked seed	300	95,7	89,7	76,5 ± 1,16	3,8 ± 0,17
»	3.900 » , » »	450	93,3	63,8	49,1 ± 1,63	3,9 ± 0,15
»	5.800 » , » »	600	84,0	20.7	37,4 ± 1,68	6,5 ± 0,33
»	9.300 » , » »	600	52,7	14,0	30,5 ± 1,47	4,1 ± 0,30
»	13.900 » , » »	750	31,2	4,0	21,1 ± 2,93	4,5 ± 1,58

experiments with doses up to 50.000 *dis* it has been found that the survival curve is of the expected sigmoid type but with a slight steep at the inflexion point. The X_1 data on percentage of seedlings and mature plants give more pronounced sigmoid curves in good accordance with earlier investigations (MÜNTZING, 1941; GUSTAFSSON, 1942, 1947 a; FREISLEBEN and LEIN, 1943 a). That the killing of dormant seed by neutrons may be even less than after X-ray irradiation has earlier been confirmed by KOSTOFF (1946) for *Crepis capillaris*. His explanation that the lower efficiency should be due to the poorer penetration of the neutrons through the seed coat cannot be accepted. Against such an interpretation *i. a.* the results from pre-soaked seed are eloquent. The observation of GRAY and READ (1943) that the relative efficiency of a radiation compared with another increases more rapidly with higher ion density for tumour than for normal tissue is certainly directly comparable with the above finding.

If the number of hits necessary to cause the biological events registered here by the death be low, the obtained results could be expected from the target-theory point of view. But the different spatial distribution of ionizations after X-rays and neutrons also allows for an explanation in accordance with the indirect action hypothesis including inactivation of substances indispensable for life.

Effects on viability. — The effect of neutron irradiation was found to be more uniform, *i. e.* showed a less pronounced biological variation within each dosage, than in the case of X-rays. This result is interesting, as a similar observation was made after treatments with atomic bomb radiations (ANDERSON, LONGLEY, LI, and RETHERFORD, 1949; RANDOLPH, 1950).

During the vegetation period a pronounced difference in the development of X_1 and N_1 plants could be further observed. Even after the highest doses of X-rays the surviving plants showed up a normal vegetative appearance, while the N_1 plants, at least after the higher doses, looked weak and unhealthy. If the tillering capacity, in Table 1 given as the mean number of spikes per plant, after the higher doses of X-rays and neutrons be compared, this difference becomes clear, as the thin stand due to the low survival here permits the plants to tiller more at liberty. The same difference could also be illustrated by another viability character, the mean number of spikelets per ear. If only the 1949 experiment with dormant seed be considered and all the treatments of each radiation be bulked together, it will give the following results:

	X-rays	Neutrons
Mean No. of spikelets per ear on the total	27,19 ± 0,101	25,10 ± 0,101
Ditto per dose, given by range of variation	25,1—29,1	23,6—26,0
Total No. of ears examined	1732	1776

This lower viability of the N_1 plants is surely a direct effect of the genetic disturbances caused by the irradiation of the seed, as the found inter-relation fits very well with the observed fertility in N_1 and X_1, respectively.

GELIN (1941) has found a pronounced correlation between the chromosome disturbances observed in the first mitotic cycles after germination of irradiated seed and the zygotic sterility observed in the X_1 generation. Due to the favourable morphology of the spike of two-rowed, selfing barley the zygotic fertility or the seed setting ability has since then often been used as an indication of genetic disturbances (GUSTAFSSON and co-workers, 1940, 1942, 1946 b, 1947 a, 1948 b, 1950; FREISLEBEN and LEIN, 1943 a; KAPLAN, 1949). It should, however, be pointed out that regeneration or substitution of killed or weakened cells or shoots together with the selective effect on the different types of chromosome aberrations by a high number of successive mitoses considerably change the original picture. Another complicating circumstance is the pronounced chimaera character of all affected X_1 and N_1 plants, due to the above-mentioned phenomena and the multiplicity of the independent primordia of each seed.

If all these conditions be taken into account, it may be explicable why the efficiency of the neutrons over the X-rays, as judged by the plant fertility, can be changed in comparison to that found for cytological disturbances immediately after germination, *viz.* to a *dis/r* efficiency of about 4 against that of 0,6 previously found (N/X ratio = 68 against 10). This extremely high relative efficiency of neutrons to produce sterility in barley is higher than in any other experiment performed and must again be explained by the specific characteristics of dormant cells. The ratio has been calculated as the mean of the results presented in Table 1 for all the three years. Even if the 1947 experiment shows up very low figures on the whole, it agrees very well with the others, showing a nearly linear relationship both for X-rays and neutrons at lower doses and a saturation effect at higher ones [1]. This decreasing

[1] The fact that the plant fertility of the untreated controls can vary with a difference of at least 10 per cent is certainly due to more or less suitable weather conditions around the time of fertilization, an observation of great interest from an agronomic standpoint. Even if a high tillering and a favourable development of the

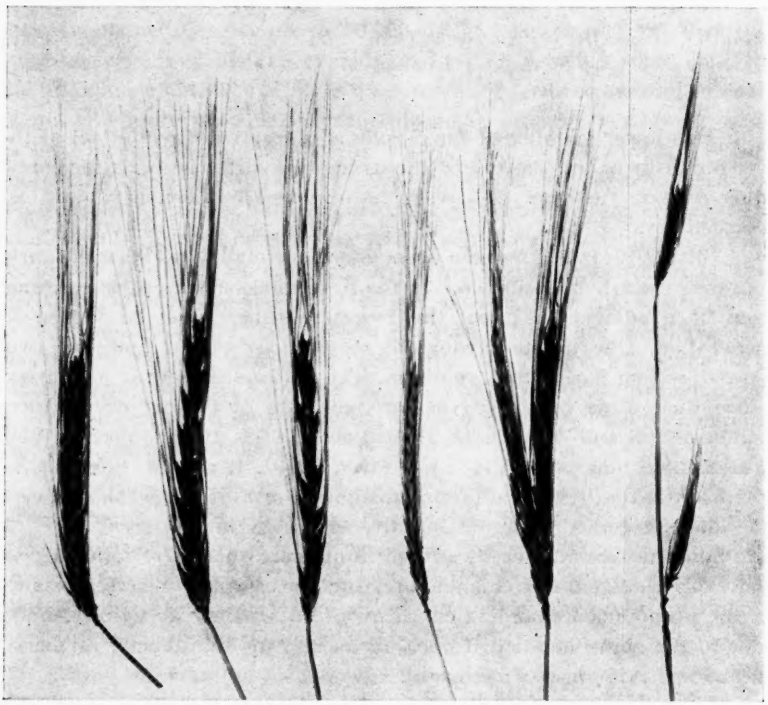

Fig. 3. Spike No. 1 from the left is of an untreated control. Nos. 2—4 show different degrees of spike sterility in the first generation after irradiating barley seeds. Nos. 5—6 are bifurcated spikes observed in X_1 and N_1, indicating irregularities of the growing point.

efficiency of successively added quantities of irradiation is especially evident after X-rays, where the fertility/dose curve soon becomes horizontal (*cf.* Table 1 and Fig. 3). From a purely genetical standpoint there are no reasons why the plant fertility of X_1 should not be able to fall down to that obtainable in N_1, if only high enough doses were applied. From several investigations it is, however, known that X-rays, besides the genetical effect, also induce direct physiological reactions of

spikes have occurred, the grain yield consequently can decrease appreciably, even if a certain compensation, due to a higher grain weight, is to be expected. In other material a decreased fertility has been observed after late sowing or after attacks of powdery mildew.

high toxicity to the cell (*cf.*, *e. g.*, SAX and SWANSON, 1941). Due to the difference in ion distribution in the cell after X-ray and neutron irradiation and the lower efficiency of the former to break a chromosome, it may be expected that a higher physiological affection should be paralleled with a certain induced frequency of chromosome disturbances in X_1 than in N_1. The threshold value for physiological reactions, able to kill the cell, will thus be reached sooner after X-rays. Due to environmental factors this threshold value will vary, but it seems to result in a minimum average plant fertility of 60—70 per cent under normal conditions. A similar inter-relation is certain also valid for neutrons. The physiological disturbances primarily induced by the irradition are, however, at least in dry seeds, where the transport of energy is at minimum, of such a subordinate importance in comparison to the genetic effects that death here is probably more an indirect consequence of these genetic irregularities. Such an interpretation also agrees with the observation of PETROVA (1942) that the lethality of α-rays on *Zygnema* cells was 700 times smaller, if death were induced via plasmatic rather than via nuclear disorganization.

That the fertility of the X_1 plants should be regulated by another factor besides the genetic disturbances is also evident, if the distribution of the single plants as to fertility be studied after increasing dosages of X-rays and neutrons, respectively. In Fig. 4 the 1949 experiment has been taken to illustrate this tendency. The distribution of the X_1 plants is skew in all doses with the highest representation in the fertile and slightly sterile classes, while the N_1 curves follow more the expected transmutation with the medians successively turning to the more sterile classes.

It must, however, be noticed that the difference in physiological effect of X-rays and neutrons is certainly not the only circumstance which can explain the higher N/X ratio found in a later stage of plant development. As LEA (1946) points out, the more densely ionizing radiation does more damage to a chromosome when breaking it. Therefore, it is to be expected that a higher proportion of neutron-induced restitutional breaks should be accompanied by minute interstitial deletions than after X-rays. As even somewhat larger intercalary deficiencies, also these difficult to detect in such a relatively unsuitable cytological material as barley, should be expected in a higher frequency after neutrons than after X-rays (KAUFMANN, 1941), a certain underestimation of the cytogenetic efficiency of neutrons relative to that of X-rays has certainly been made by a mere registration of fragments and bridges.

Besides this explanation of the higher N/X ratio observed for the plant fertility, still another additional factor may intervene. When neutron particles are slowed down by elastic collisions with the atoms of the irradiated tissue and their velocity is reduced to that of thermal energies, they are captured. The atom that has captured a neutron forms a new compound nucleus with an excess of energy. In biological material hydrogen, nitrogen, and boron should be the main elements in these capture reactions (ZIRKLE, 1947; CONGER and GILES, 1950; GRAY, 1950). Most of the excess energy is immediately emitted, but a part will

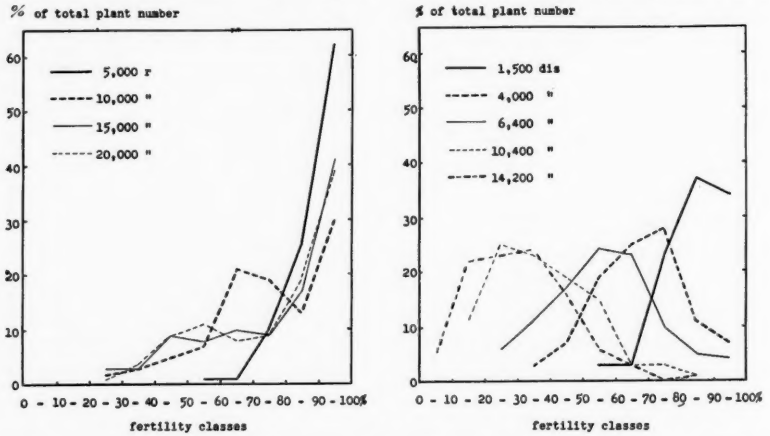

Fig. 4. Diagrams showing the different distributions of X_1 (to the left) and N_1 (to the right) plants into fertility classes at successively increasing doses. Data taken from the experiments of 1949 with dry barley seeds.

be stored and acts as a delayed radiation. As this internal radiation is more effective than the one delivered from external sources (CONGER and GILES, 1950; THOMPSON, MAC KEY, GUSTAFSSON, and EHRENBERG, 1950), even rather small quantities may be of importance. To what extent such a delayed radiation is able to increase the genetic changes caused by the immediately produced recoil nuclei is unknown, but it is impossible that it can be responsible for the whole increase of the X/N ratio found above.

Effects of pre-soaking. — A great difference in the radio-sensibility has frequently been observed, due to whether the cell is in rest or not. The increased susceptibility to roentgen irradiation by pre-soaking the seed has been studied by STADLER (1928 b, 1931), GUSTAFSSON (1936 a and b, 1940), WERTZ (1940), KNAPP and KAPLAN (1942), and D'AMATO

and GUSTAFSSON (1948). From these experiments it seems evident that already with small increases of water the cytogenetic irregularities become more numerous (GUSTAFSSON, *l. c.*) and that this fact at least to a large extent may be due to an increase of the target volume (WERTZ, *l. c.*). If dormant seeds be pre-soaked only a few hours, the increased sensibility is reversible when dehydrating the seeds again (KNAPP and KAPLAN, *l. c.*). To a certain extent the nuclear susceptibility probably

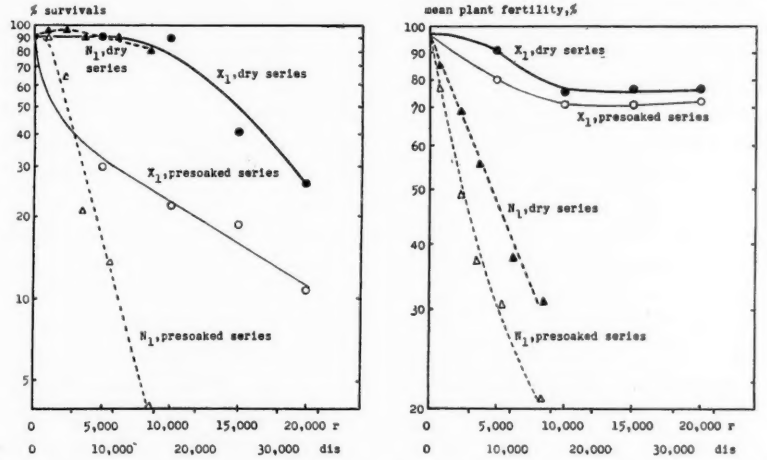

Fig. 5. Semilogarithmic diagrams giving the survival/dose curves in X_1 and N_1 of irradiated dry (black circles and triangles, respectively) and pre-soaked (white circles and triangles) barley seeds (to the left) and the corresponding fertility/dose curves of the surviving plants (to the right). Only data from the 1949 experiment used. Interradiational dose scale: 1 *dis* = 0,6 *r*.

becomes irreversible already after some 6 hours of pre-soaking, but the first mitoses do not start before 24 hours or even later (WERTZ, *l. c.*).

This lower resistance to irradiation characteristic of chromosomes in cells with increased metabolic activities is also found in the present material. If first the action of X-rays on pre-soaked seed be considered, it may be observed that the higher genetic sensibility is here paralleled by about the same increase of the physiological or killing effect. This statement is based on data given in Table 1 or Fig. 5, as the nearly unchanged X_1 sterility and the higher death-rate characteristic of the pre-soaked in comparison to the dry series can scarcely be interpreted in another way. The plant fertilities of the pre-soaked series are, however,

all through slightly lower than those of the dry series. A more intensive metabolic activity of the chromosomes to that of other parts of the cell and thus a higher radio-sensibility is, therefore, not impossible at the examined stage of germination.

That pre-soaking before irradiation increases mortality, is also valid when treating with neutrons. In their hydrated stage the seeds were even more sensitive in this respect to neutrons than to X-rays in full agreement with the earlier cited results with different fast-growing tissues. The simultaneous decrease in the fertility of the surviving plants (*cf.* Fig. 5) indicates that the higher lethality observed after pre-soaking the seed cannot be explained only on a physiological basis as was possible in the X-ray experiments. In analogy to the experience from dry seeds the lethal effect of neutrons on pre-soaked seed must be more an indirect consequence of the induced chromosomal disturbances than a mere breakdown independent of this influence on mitosis.

This opinion that the physiological killing effect of neutrons should be of subordinate importance, while it should play a great rôle in connection with X-raying, is in conflict with the opposite view of SPEAR and TANSLEY (1944) based on inhibition of mitosis in rat retina by γ-rays and neutrons. The tendency of their data is, however, not conclusive and their interpretation has more the form of a discussion than a direct statement. It may be pointed out that an ignored coincidence between the use of slow-growing rat retinas (English race) in the γ-ray experiment and the high-retarding effect on mitosis typical for this radiation against the fast-growing retinas of American rats used in the neutron experiment, where the retarding effect at comparable tissue doses was much smaller, may endanger the conclusions.

When considering the very pronounced difference in susceptibility between neutron irradiated dry and pre-soaked seed series, it ought to be kept in mind that the energy absorption from a transmitted neutron beam is certainly higher in the pre-soaked seed, due to the high moderating effect of water. To what extent this circumstance has influenced the result, is impossible to say at present. There is, however, reason to believe that this higher energy absorption per unit volume of tissue plays a very important rôle, if the higher effect of pre-soaking in N_1 in comparison to X_1 be considered. The other intervening factor, the increased sensibility of the chromosomes when entering into division, must, namely, be less important to neutrons than to X-rays, due to the high spatial energy concentration of the former type of radiation. GILES (1943) has, however, proved that this factor is not completely negligible

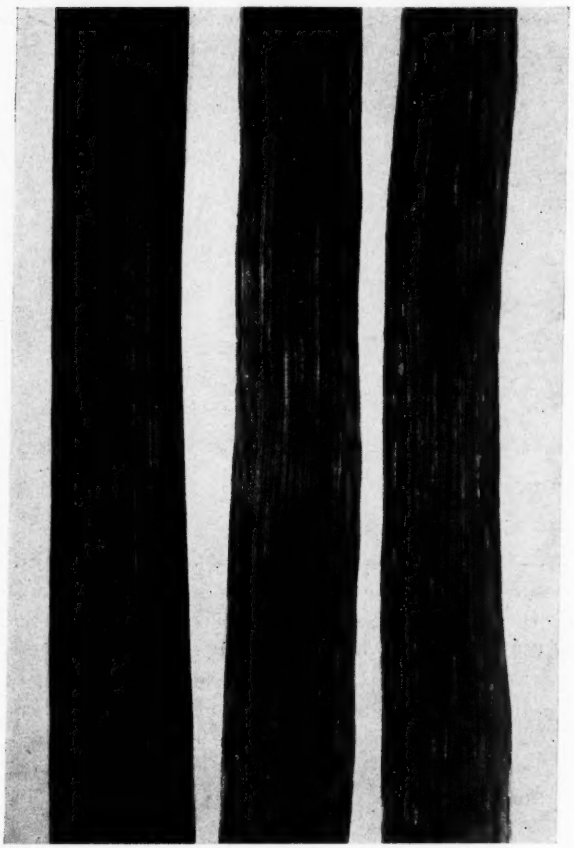

Fig. 6. Control leaf to the left. To the right two mottled leaves of seedlings from neutron-irradiated barley seeds. The flecking is due to induced degenerations of the plastids.

in neutron treatments, as the protons produced by fast neutrons were also shown to have a probability less than unity to break a transversed chromatid.

If the problem be considered from all possible sides, the biological buffering should also be mentioned, as DALE (1940, 1942, 1943) and especially FORSSBERG (1945, 1946, 1947, 1950) have found that unsaturated substances with high affinity to the activated water have a very pronounced protection effect. This buffering certainly increases,

TABLE 2. *The induced frequency of striata chimaeras and bifurcated spikes in X_1 and N_1.*

Year	Treatment	No. of plants examined	Frequency of *striata* chimaeras		Frequency of bifurcated spikes	
			abs.	rel.	abs.	rel.
1947	Control	251	—	0,0	—	0,0
1948	»	551	—	0,0	—	0,0
1949	»	687	—	0,0	—	0,0
	Total:	1489	—	0,0	—	0,0
1947	X-rays	523	—	0,0	5	1,0
1948	»	645	2	0,3	13	2,0
1949	»	1126	4	0,4	4	0,4
	Total:	2294	6	0,3	22	1,0
1947	Neutrons	1043	15	1,4	18	1,7
1948	»	1075	8	0,7	27	2,5
1949	»	1716	64	3,7	34	2,0
	Total:	3834	87	2,3	79	2,1

when the seed becomes soaked, thus counterbalancing the dilution effect or — to use another expression — the enlargement of the sensitive target volume. As the protection phenomenon must be less apparent in connection with neutrons than with X-rays, again due to the higher energy concentration of the former type of radiation, there could here be another reason for the conspicuous effect of neutrons on pre-soaked seeds. The data from the X-ray experiments show, however, that this phenomenon must be of much less importance than the dilution effect when considering the influence of soaking.

Other first generation effects. — Besides the above-mentioned phenomena observed in the first generation some other abnormalities of certain interest have been found. In accordance with the experiments on atomic bomb radiations performed by ANDERSON, LONGLEY, LI and RETHERFORD (1949), RANDOLPH (1950), and SMITH (1950), the first leaves of especially the N_1 seedlings were characterized by an even distributed mottling and streaking in an intensity proportional to the dose (Fig. 6). This »flecking» in the chlorophyll was more easily observable in greenhouse conditions than outdoors and successively disappeared. As in the above cited experiments yellow or white stripes or sectors on the leaves were occasionally observed at later stages. In Table 2 the frequency of such *striata* chimaeras are bulked, giving no statistical significance between the X-ray material and the control. Neutrons gave a

definitely higher frequency ($\chi^2 = 32{,}95$ and $23{,}59$ as against control and X-ray material, respectively; $P < 0{,}001$). The χ^2-analysis has been performed after a correction for discontinuity elaborated for 2×2 tables (*cf.* BONNIER and TEDIN, 1940). As no chimaeras of the first generation gave rise to chlorophyll mutants in X_2 and N_2 with the same change of colour, all speculations as to whether there was a plasmatic, dominant or recessive inheritance must be conjectural.

Another first generation effect of irradiation was the induction of bifurcated spikes (Fig. 3). This abnormality has earlier been found in X-rayed barley by FREISLEBEN and LEIN (1943 a), who interpreted the phenomenon as connected with a regeneration of an affected meristem. In Table 2 the number of such bifurcated spikes observed in the different materials are presented. A χ^2-analysis proves that both X-rays ($\chi^2 = 12{,}75$; $P < 0{,}001$) and neutrons ($\chi^2 = 29{,}71$; $P < 0{,}001$) are responsible for this type of abnormality and that this phenomenon is more frequent after neutrons than after X-rays ($\chi^2 = 6{,}36$; $P = 0{,}01$). 81 of these bifurcated spikes were progeny-tested. As none was able to inherit this special characteristic and 4,9 per cent gave chlorophyll mutations as against 5,5 per cent in the total material, the phenomenon probably has no directly genetical background. It is of interest that a similar effect has been obtained by irradiation of fruit trees, *viz.* a bifurcation of shoots and leaf midribs (GRANHALL, GUSTAFSSON, NILSSON, and OLDÉN, 1949).

RATES AND TYPES OF CHLOROPHYLL MUTATIONS INDUCED IN THE SECOND GENERATION.

When discussing the frequency of induced chlorophyll mutations, it must be pointed out that the irradiated object, the barley seed, offers some difficulties in giving a correct basis for calculations. The ideal unit on which the mutation rates ought to be based, is the surviving spike primordium. Owing to lack of information on the degree of development, substitution, or competition of the different primordia within one embryo, it is impossible to obtain such a basis for the calculations. As a substitute the spike as unit has been proposed by STADLER (1928 a) and later on preferred also by GUSTAFSSON (1940), SMITH (1942), FREISLEBEN and LEIN (1943 b), and KAPLAN (1949). If only one spike per plant is tested, this mode of calculation is correct. If all spikes of each plant be counted in, a systematic error will intervene. This depends on the facts that only a very low percentage of the mutated plants have more than one affected spike (X_1: 6,2 per cent; N_1: 6,5 per cent) and that

TABLE 3. *Rates of chlorophyll mutations in the second generation of irradiated barley.*

Year	Treatment	No. of examined plant progenies	No. of examined spike progenies	No. of examined plants per spike progeny	Germinability % 2nd gen.	No. of chlorophyll mutations	Mutation rate per plant progeny	Mutation rate per spike progeny	Mutation rate per dose unit and plant progeny, $\cdot 10^{-5}$	Mutation rate per dose unit and spike progeny, $\cdot 10^{-5}$
1947	Control, dry seed	251	820	19,8	95,4	1	0,4	0,1	—	—
1948	» , » »	551	1983	23,3	97,2	2	0,4	0,1	—	—
1949	» , » »	500	1807	24,6	97,5	5	1,0	0,3	—	—
1947	5.000 *r*, » »	215	635	15,1	92,1	28	13,0	4,4	2,6	0,9
»	10.000 », » »	156	527	11,2	86,0	31	19,9	5,9	2,0	0,6
»	15.000 », » »	60	293	13,3	85,3	10	16,7	3,4	1,1	0,2
»	20.000 », » »	51	238	13,0	84,8	10	19,6	4,2	1,0	0,2
»	25.000 », » »	30	143	12,3	83,0	8	26,7	5,6	1,1	0,2
1948	10.000 », » »	215	712	18,2	95,3	35	16,3	4,9	1,6	0,5
»	15.000 », » »	215	706	17,6	94,8	49	22,8	6,9	1,5	0,5
»	20.000 », » »	215	771	16,1	91,8	43	20,0	5,6	1,0	0,3
1949	5.000 », » »	100	318	22,2	94,3	11	11,0	3,5	2,2	0,7
»	10.000 », » »	100	312	18,6	93,6	18	18,0	5,8	1,8	0,6
»	15.000 », » »	100	354	21,1	94,9	18	18,0	5,1	1,2	0,3
»	20.000 », » »	100	441	23,0	94,2	15	15,0	3,4	0,8	0,2
»	5.000 », soaked seed	100	443	22,6	95,0	13	13,0	2,9	2,6	0,6
»	10.000 », » »	100	443	22,5	92,5	11	11,0	2,5	1,1	0,2
	15.000 » » »	100	449	20,6	89,1	22	22,0	4,9	1,5	0,3

»	10.000 », » »	100	443	22,5	92,5	11	11,0	2,5	1,1	0,2
»	15.000 », » »	100	449	20,6	89,1	22	22,0	4,9	1,5	0,3
»	20.000 », » »	100	458	22,8	92,0	17	17,0	3,7	0,9	0,2
1947	2.500 *dis*, dry seed	215	700	14,7	92,9	33	15,3	4,7	6,1	1,9
»	4.500 », » »	215	714	12,0	88,7	42	19,5	5,9	4,3	1,3
»	7.000 », » »	214	678	9,1	83,7	49	22,9	7,2	3,3	1,0
»	9.000 », » »	202	677	7,2	79,6	40	19,8	5,9	2,2	0,7
»	13.500 », » »	135	375	6,3	74,0	37	27,4	9,9	2,7	0,7
1948	1.000 », » »	215	709	19,8	97,3	13	6,0	1,8	6,0	1,8
»	1.300 », » »	215	710	18,3	96,5	26	12,1	3,7	9,3	2,8
»	3.300 », » »	215	691	11,9	90,2	47	21,9	6,8	6,6	2,1
»	5.700 », » »	215	629	12,3	90,9	42	19,5	6,7	3,4	1,2
»	6.100 », » »	215	710	10,3	90,9	52	24,2	7,3	4,0	1,2
1949	1.500 », » »	100	369	20,9	94,1	14	14,0	3,8	9,3	2,5
»	4.000 », » »	100	345	16,1	91,0	19	19,0	5,5	4,8	1,4
»	6.400 », » »	100	283	12,7	90,4	18	18,0	6,4	2,8	1,0
»	10.400 », » »	100	281	9,2	85,7	21	21,0	7,5	2,0	0,7
»	14.200 », » »	100	263	7,4	75,0	25	25,0	9,5	1,8	0,7
»	1.400 », soaked seed	100	351	18,3	93,1	16	16,0	4,6	11,4	3,3
»	3.900 », » »	100	339	11,8	89,6	24	24,0	7,1	6,2	1,8
»	5.800 », » »	100	395	11,6	80,9	38	38,0	9,6	6,6	1,7
»	9.300 », » »	84	229	8,9	71,2	16	19,0	7,0	2,0	0,8
»	13.900 », » »	27	77	9,6	86,5	10	37,0	13,0	2,7	0,9

there exists a correlation between higher dose, higher lethality, thinner stand, and thus better tillering of surviving plants (*cf.* Table 1). This consequence has earlier often been overlooked, resulting in a certain underestimation of the mutation frequency at higher doses. The phenomenon can be studied in Table 3 from which it is also evident that the X-ray figures are more influenced than those of neutrons. Due to this difference, it is certainly more acceptable to base the mutation rates on the plant as unit. In all discussions about type of mutation and its association to a certain X_1 or N_1 sterility, the spike is, however, the more appropriate unit. Here the difference in tillering will give the plant unit a disadvantage.

There is also another circumstance that must be considered in connection with an evaluation of the obtained rates of mutation. The plant or spike fertility decreases with the dose and this more obviously after neutrons than after X-rays. A decreased number of grains disposable for the second generation test means a smaller chance of a recessive mutation being registered, especially as the mutation, as shown above, is generally induced in only one spike of the mutated X_1 or N_1 plant. A lower fertility is accompanied by a lower germinability (*cf.* Table 3) which implies a further underestimation of the real mutation frequency at higher doses. It is, however, impossible to make any acceptable corrections for these phenomena. To give an idea about their influence on the results, the mean number of examined X_2 or N_2 plants per spike progeny is inserted in Table 3. If these values be compared with the average normal/mutant ratio of $6{,}6 \pm 0{,}19 : 1$ found in the present study, it will be obvious that controls and low dosages give a probability near unity to observe all the induced mutations. Especially at high neutron treatments the probability may, however, fall to 0,7 or even less. The circumstance that the theoretically expected 3 : 1 ratio was not obtained, is explained by the chimaeric structure and complexity of affection in many spikes as well as an inferior viability of the chlorophyll mutants.

In order to exclude all confusions it may be mentioned that mutant plants of identical appearance but observed in different spike progenies of one X_1 or N_1 plant are estimated as one case of mutation both when plant and spike have been used as unit.

Mutation rates. — It has been pointed out before that the dose range applied in the present experiments has been chosen very high for practical reasons. This leads to the consequence that only the upper part of the frequency/dose curves can be examined (Table 3 and Fig. 7). Here, however, a saturation effect sets in together with a contra-selection

due to high lethality, which makes the material less suitable for more detailed calculations. If target theoretical aspects be laid on the results, graphically illustrated in Fig. 7 with the cytogenetically obtained 1 *dis* = 0,6 *r* as basis for a biological comparison of neutron and X-ray dosages, the induction of chlorophyll mutation must be considered to

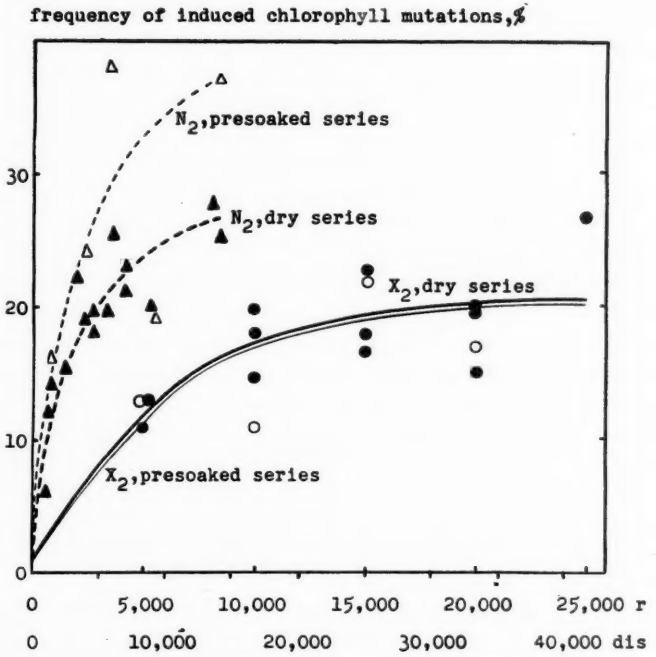

Fig. 7. Diagram giving the frequency/dose curves for induced chlorophyll mutations in the X_2 and N_2 generation of irradiated dry (black circles and triangles, respectively) and pre-soaked (white circles and triangles) barley seed. Inter-radiational dose scale: 1 *dis* = 0,6 *r*.

be mostly 1-hit events. This is to be expected, as this type of mutations corresponds to recessive lethals of *Drosophila* in many ways (GUSTAFSSON and MAC KEY, 1948 b). If this comparison holds, it is interesting to state that neutron treatment both of dry and pre-soaked seed has been found more effective than X-rays in producing these definite biological events, and that this result is directly opposite to that obtained for recessive lethals of *Drosophila* (TIMOFÉEFF-RESSOVSKY and ZIMMER,

1938; DEMPSTER, 1941; GILES, 1943; FANO, 1944). This inconsistency may certainly be explicable, if the difference in irradiation resistance and dose range be taken into consideration. Both recessive lethals of *Drosophila* (*cf.* LEA, 1946; CATCHESIDE, 1948) and chlorophyll mutations of barley are inhomogeneous groups, including true gene mutations, deletions and interchanges. As FANO (1944) has pointed out and proved for *Drosophila* lethals, the relative frequency of the three types of genetic mechanisms underlying the discussed kind of mutations is not the same in X-ray as in neutron experiments. The last-mentioned radiation is more effective in producing chromosomal re-arrangements but less effective in producing »gene» mutations than the former. From this point of view it is evident that the N/X efficiency is largely dependent on the extent, at which induced deletions and interchanges can be carried through the heterozygous stage. As this capacity is definitely higher for barley than for *Drosophila* flies, it certainly means that a considerable amount of gross structural changes are associated with chlorophyll mutations in barley, while those in *Drosophila* will be classified as dominant lethals, a group which is known to have an N/X ratio above 1 (DEMPSTER, 1941; FANO, 1943). This circumstance together with the pronounced contra-selection at the high X-ray dosage applied in the present experiments is certainly enough to explain this apparent contradiction.

The identical course of the frequency/dose curves for dry and presoaked X-ray materials, which is not obtained when treating with neutrons, again emphasizes the more pronounced physiological effect of X-rays (*cf.* Table 3 and Fig. 7).

The assumption that the artificially induced chlorophyll mutations of barley should to a great extent be associated with structural changes has been earlier proved by GUSTAFSSON (1940) and GELIN (1941), and later verified by FREISLEBEN and LEIN (1943 b). That the same conclusion must be drawn from the present results, can also be proved by grouping the material into fertility classes and determining the degree of heterogeneity between mutated and non-mutated spikes. The grouping of the material into such classes is made from genetical aspects and agrees in most respects with that proposed by GUSTAFSSON (1940). The condensed X-ray data give the following distribution:

	Fertility of X_1 spikes					
	0 — 10	— 70	— 90	— 100	— 100 %	
No. of non-mutated X_1 spikes	94	2302	1398	1845	1242	= 6881
Expected	89,30	2338,95	1413,63	1816,44	1222,68	

	Fertility of X_1 spikes					
	0 — 10	— 70	— 90	— 100	— 100 %	
No. of mutated X_1 spikes	—	160	90	67	45	= 362
Expected	4,70	123,05	74,37	95,56	64,32	

$\chi^2 = 35{,}18$; d. f. = 4; P-value < 0,001

The corresponding neutron data are:

	Fertility of N_1 spikes					
	0 — 10	— 70	— 90	— 100	— 100 %	
No. of non-mutated N_1 spikes	417	4957	1568	1221	739	= 8902
Expected	401,88	4964,56	1608,43	1201,89	725,24	
No. of mutated N_1 spikes	13	355	153	65	37	= 623
Expected	28,12	347,44	112,57	84,11	50,76	

$\chi^2 = 33{,}05$; d. f. = 4; P-value < 0,001

From these tests of heterogeneity it is evident that the chlorophyll mutations are concentrated to the partially sterile spikes. That the underrepresentation in the sterile class should be due to other concomitants than that of a too low number of second generation plants examined (1—3 plants per progeny), is difficult to state. The fact that the slightly sterile (70—90 %) N_1 spikes show a relatively higher mutation frequency in comparison to the 10—70 % group as judged from the total material may indicate that chlorophyll mutations are generally not associated with too drastic genetic disturbances.

In full conformity with the findings of FANO (1944) for recessive lethals of *Drosophila*, the neutron induced chlorophyll mutations of barley are more associated with gross chromosomal re-arrangements than in the X-ray material. If this fact, on the other hand, should mean that X-rays are producing a higher proportion of true gene mutations than neutrons, is more difficult to state. The fertile mutations may to a great extent be due to restitutional breaks, *i. e.* they can be loss mutations. As the restitution certainly is less common after neutron than after X-ray irradiation (*cf.* LEA, 1946), the data given below can just as well be considered from these aspects.

	Fertility of X_1 and N_1 spikes					
	0 — 10	— 70	— 90	— 100	— 100 %	
No. of mutated X_1 spikes	—	160	90	67	45	= 362
Expected	4,78	189,27	89,30	48,51	30,14	
No. of mutated N_1 spikes	13	355	153	65	37	= 623
Expected	8,22	325,73	153,69	83,49	51,87	

$\chi^2 = 37{,}98$; d. f. = 4; P-value < 0,001

Another problem of interest is the possibility of a non-random distribution of induced mutations after neutron irradiation, found by NAGAI and LOCHER (1938) and NISHINA and MORIWAKI (1939, 1941), but denied by FANO (1943, 1944). No such tendency has been found in the present material. The X_1 spikes segregating one type of chlorophyll mutation were 4,83 per cent and those giving rise to two independent types 0,08 per cent of the total material, as against an expected frequency of 0,23 per cent. The corresponding N_1 data were 6,08, 0,21, and 0,37 per cent, *i. e.* just about the same under-representation of spikes with two different mutations.

Types of chlorophyll mutations. — The numerous genotypic changes giving rise to chlorophyll mutations correspond to a rather limited number of superficially uniform phenotypes. This circumstance has led GUSTAFSSON (1940) to introduce a practical system of classifying the different chlorophyll mutations at seedling stage. When his grouping is used as basis for a genetical test of the mutation process, it must, however, be remembered that the groups are genotypically more or less heterogeneous. This diminishes their value, if they be used to verify a direction of the mutation process caused by various treatments. A significant difference in the relative commonness of the different phenotypes may mean a real difference. If no such tendency occurs, it is, how-

TABLE 4. *Types of chlorophyll mutations and their absolute frequencies in the X_2 and N_2 generation of barley.*

Year	Treatment	No. of chlorophyll mutations observed					
		Albina	*Viridis*	*Albo-viridis*	*Xantha*	Others	Total
1947	X-rays, dry seed	40	28	5	8	6	87
1948	» , » »	54	49	9	6	9	127
1949	» , » »	29	24	2	3	4	62
	Σ	123	101	16	17	19	276
1949	» , soaked seed	27	24	7	3	2	63
	X_2, total:	150	125	23	20	21	339
1947	Neutrons, dry seed	104	61	10	12	14	201
1948	» , » »	100	48	9	12	11	180
1949	» , » »	39	32	12	4	10	97
	Σ	243	141	31	28	35	478
1949	» , soaked seed	43	37	11	7	6	104
	N_2, total:	286	178	42	35	41	582
	X_2 and N_2, total:	436	303	65	55	62	921

ever, more dangerous to conclude that the mechanism underlying the mutagenic effect of the compared agents should therefore be identical.

This fact must be considered in the present study, where no difference has been observed between X-rays and neutrons in their ability to induce various chlorophyll mutations and this in spite of their different ability to produce gross chromosomal re-arrangements.

In Table 4 the obtained data are presented in a very condensed form, as a thorough analysis has not revealed any difference in the distribution of the mutations due to the size of dose. A test for an eventual heterogeneity in the material presented will give the following results:

	χ^2	d. f.	P-value
Dry contra pre-soaked seed, X_2 and N_2	5,84	9[1]	0,75
1947: X_2 contra N_2	1,44	4	0,82
1948: » » »	7,00	4	0,34
1949: » » »	3,29	4	0,50
Total: » » »	6,35	6[2]	0,39
X_2, between years	3,83	8	0,87
N_2 » »	14,72	8	0,07

[1] *Xantha* and »others» bulked together.

[2] »Others» divided up in three groups: *tigrina*, *striata* and »others».

In apparent disagreement with earlier X-ray experiments on pre-soaked seed, no relative increase of rare mutations, especially those of *alboxantha* type, has been found in the present material (*cf.* GUSTAFSSON and co-workers, 1940, 1947 a, 1948, 1949). As the X_2 data for pre-soaked seed are limited in the present case, it may be difficult to argue in any special direction. It appears, however, as if this special effect of hydration sets in first at a certain stage of the germination which is probably determined by the beginning of the nuclear divisions. In the present case the pre-soaking may not have been effective enough to force the seed material to that certain point before the irradiation was applied.

As a further comment to the given heterogeneity tests, it may be pointed out that both the years 1947 and 1948 show a relatively higher proportion of *albinas* in N_2 than in X_2. This has not been verified in 1949, resulting in a rather low P-value for »between years». This difference, though not statistically significant, can hardly be explained otherwise than suggesting physiological variations, thus illustrating the danger of drawing conclusions from one single set of experiments only.

It is interesting to state that SMITH (1950) found the same lack of

striking differences in the kinds of chlorophyll mutations obtained when comparing X-rays and atomic bomb radiations.

First generation fertility and type of mutation. — Above it has been pointed out that artificially induced chlorophyll mutations to a great extent were associated with chromosomal re-arrangements. To what extent the different types of these mutations are connected with certain chromosome disturbances is under discussion. GUSTAFSSON (1940) maintains that *xantha* types are concentrated to the offspring of distinctly but slightly sterile X_1 plants and that *viridis* types show an even higher dependence on X_1 sterility, while *albina* types will more readily be found in all fertility classes. Such correlations between degrees of sterility in X_1 and different categories of mutation in X_2 have, however, not been found by MÜNTZING (1942) or FREISLEBEN and LEIN (1943 b). This forced GUSTAFSSON (1946 b, 1947 a) to a critical analysis of their data, and at least for the *xantha* types he was able to give a convincing demonstration of their association with a certain degree of X_1 sterility.

Owing to these conflicting opinions it may be of a certain interest to examine the present material also from this point of view. This all the more as it has been treated in exactly the same manner as that of GUSTAFSSON. To allow as detailed a grouping as possible and thus to increase the chance of detecting any existing dependence, a bulking of the whole material is to be preferred, if biologically permitted. As the mutation curves as well as the affection curves (see below) indicate point events, there are biological reasons for permitting such a bulking of plants from different treatments. The circumstance that there is no tendency to different relative frequencies of the special phenotypes after high or low dosages also strengthens the correctness of such a bulking.

A more direct estimation of the possibility of considering the total material, *i. e.* both X_1 and N_1 together, can be obtained, if the correlation between the mean plant fertilities of the first generation and the rates of induced mutations be examined. This has been done in Table 5, showing that the fertility as a biological unit in the present comparison is just as reliable as the physical ones. That the correlation is strongest in the neutron-treated material is in full harmony with the above discussion on the connection between chlorophyll mutations and chromosomal aberrations. These high correlations obtained in X_1 as well as in N_1 justify bulking the material of both treatments and examining it from the inter-relation between degree of sterility and rates of induced chlorophyll mutation. Such an analysis has also been made in Table 5, resulting in such good conformity that at least no statistical objections

TABLE 5. *Analyses of co-variance for dose of irradiation, plant fertility of first generation and frequency of induced chlorophyll mutations in barley (dry series).*

Comparison	Degrees of freedom	Regression coeff.	Correlation coeff.
Dose—mutation frequency of 2nd gen.			
X-ray material (1947—49)	10	+ 0,00048	0,71 ± 0,211
Neutron material (1947—49)	13	+ 0,00104	0,80 ± 0,361
Plant fertility of 1st gen.—mutation frequency of 2nd gen.			
X-ray material (1947—49)	10	— 0,17	0,66 ± 0,083
Neutron material (1947—49)	13	— 0,22	0,87 ± 0,071
X-ray and neutron material (1947)	8	— 0,22	0,81 ± 0,095
» » » » (1948)	6	— 0,38	0,84 ± 0,183
» » » » (1949)	7	— 0,18	0,90 ± 0,073
» » » » (between years and radiations)	4	— 0,12	0,83 ± 0,072
» » » » (within years and radiations)	20	— 0,25	0,83 ± 0,068
» » » » (1947—49)	25	— 0,20	0,79 ± 0,050

can be made against a bulking of the X-ray and neutron material. As separate χ^2-analyses strengthen this conclusion, the material will be given here in its most condensed form.

With the spike as the most appropriate unit the distribution of the different categories of chlorophyll mutations into fertility classes gives the following result:

No. of X_1 and N_1 spike progenies segregating	Fertility of X_1 and N_1 spikes										
	0 — 20	— 30	— 40	— 50	— 60	— 70	— 80	— 90	— 100	— 100 %	
Albina	34	31	42	57	47	55	50	57	62	41	= 476
Expected	35,76	28,51	46,39	46,88	44,94	52,67	58,47	58,96	63,79	39,63	
Viridis	25	16	34	25	27	33	43	38	51	28	= 320
Expected	24,04	19,17	31,19	31,51	30,21	35,41	39,31	39,64	42,88	26,64	
Alboviridis .	6	5	5	1	7	10	13	9	8	5	= 69
Expected	5,18	4,13	6,72	6,79	6,52	7,64	8,48	8,55	9,25	5,74	
Xantha	4	1	7	6	7	6	5	12	7	1	= 56
Expected	4,21	3,35	5,46	5,51	5,29	6,20	6,88	6,94	7,50	4,66	
Others	5	6	8	8	5	5	10	6	4	7	= 64
Expected	4,81	3,83	6,24	6,30	6,04	7,08	7,86	7,93	8,58	5,33	

$\chi^2 = 35{,}14$; d. f. = 36; P-value = 0,96

Thus, no obvious tendencies have been observed in the present material indicating that a certain type of chlorophyll mutation really should be associated with a certain degree of sterility induced in the first generation of irradiated barley. The same result will be obtained, if the whole plant be used as unit ($\chi^2 = 24{,}19$; d. f. $= 32$; P-value $= 0{,}89$). Such a finding could also be expected from the idea of randomized distribution of induced mutations, if the different chlorophyll mutations were ultimately due to inactivation of different single genes. That *albina* and *viridis* types are common, *alboviridis* and *xantha* less common, and *tigrina*, *striata* and *maculata* rare, is certainly explicable by the assumption that there are different numbers of genotypes brought together in the different phenotypically specified groups. Such an interpretation is in harmony with the great variation within the *albina* and *viridis* groups and the more uniform appearance within the rare types of mutation.

If it be true that certain categories of chlorophyll mutations are concentrated to more or less sterile plants, *i. e.* they should be associated with some type or types of chromosome aberration, it should also be expected that the deviation from the 3 : 1 ratio should be more pronounced the more drastic the genetic disturbances were. A calculation of the relative proportion of normal and mutant plants in the offspring of mutated X_1 and N_1 spikes does not reveal any such clear difference between the groups:

	Normal/mutant ratio
Albina	6,49 ± 0,836 (476)
Viridis	6,83 ± 0,349 (320)
Alboviridis	6,16 ± 0,634 (69)
Xantha	6,71 ± 0,896 (56)
Others	6,56 ± 0,735 (64)
Total	6,60 ± 0,189 (985)

If thus no statistically significant evidence for a non-randomized distribution of the separate mutations between different classes of fertility could be found in the present material, it is, however, interesting to observe that the *xantha* types indicate a similar behaviour to those in the material of GUSTAFSSON (*l. c.*). The reason why one author obtains a close inter-relationship but not another may be due to different dosages used, as a relatively low representation of plants or spikes in the fully fertile classes may fog the picture. This risk obviously exists in the present material as well as in the materials of MÜNTZING (*l. c.*) and FREISLEBEN and LEIN (*l. c.*).

When discussing these questions it should also be pointed out that the decreased sterility of the first generation certainly only gives a rather approximate idea of the direct mechanism behind an induced mutation. At least at the high doses here applied, the decrease in fertility could just as well be a summation effect due to several hits in the same cell as a direct indication of what kind of process was responsible for the observed chlorophyll mutation. That the use of plant or spike fertilities under such circumstances must be made with a certain cautiousness is also indicated from an analysis of co-variance between the fertility of sister spikes having the same chlorophyll mutation and thus probably the same origin. In the present material 59 such comparisons could be made giving a correlation coefficient of only $0{,}21 \pm 0{,}129$, *i. e.* no clear inter-relation at all.

DISCUSSION.

In the attempt to explain the biological effect of irradiation, hypotheses from very different starting points have emerged. If only one specific biological event has been studied, it is not astonishing that results have been found to agree with one hypothesis but apparently to disagree with another. Thus a discussion more of the *pro* and *con* type than the acceptance of several explanations has often arisen. In the last few years tendencies towards a less dogmatic interpretation of the observed effects have, however, appeared (*cf.* ALLSOPP, 1948). The studies of BONNIER and LÜNING (1949) and BONNIER, LÜNING, and PERJE (1949) indicate that the most accepted theory, the target theory, should not alone be able to explain the mutation process at very low doses. They suggest an additional effect of indirect mutagenic action which is quite in accordance with the results of STONE and co-workers (*cf.* HAAS, CLARK, WYSS, and STONE, 1950) who have proved that chemical mutagens can be produced by irradiation even from simple amino acids. Also CASPARI and STERN (1948) hint at a revision of the classical hit theory from their study of the dependence between the time factor of radiation effects and the mutation rate at low doses. Though MARSHAK's (1939) data on neutron-irradiated root tips allow for an interpretation according to the hit theory, this latter, however, cannot furnish a reasonable explanation of his finding that sometimes whole sections of a chromosome were spread out and markedly disorganized, *i. e.* phenomena related to the »pulverized» cells observed by LEVAN and TJIO (1948) after treatments with phenols.

In the present investigation the biological consequences of irradiation have been studied at several stages of the development of the treated object and two different types of radiation have been compared. If only one biological phenomenon due to the irradiation had been studied, *e. g.* the frequency of chromosome disturbances or the rate of chlorophyll mutations, no objections should have been found against a pure target-theoretical interpretation even from the present material. Now a close inter-relation between point events and physiological reactions of more

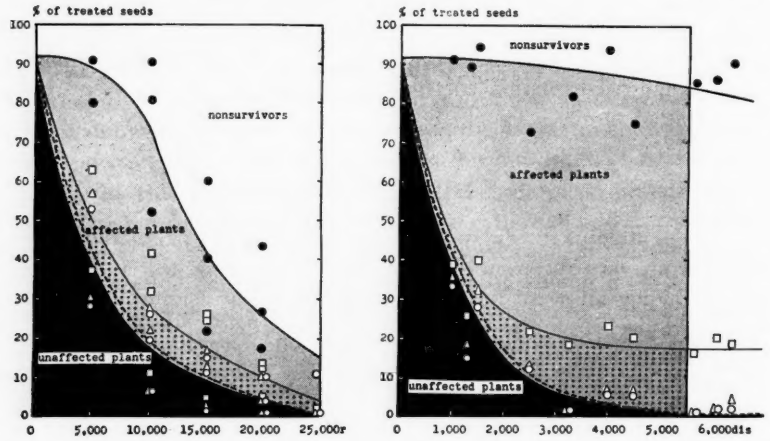

Fig. 8. Diagrams giving the relative proportions of unaffected plants (white circles and black area), affected plants (black circles and grey area) and non-survivors (white area) from the total number of irradiated dry barley seeds per dose. The dotted section of the grey area represents the relative number of plants segregating chlorophyll mutations. The broken line (white triangles) gives the boundary between fertile and partially sterile plants. X-ray experiments to the left, neutron data to the right. Inter-radiational dose scale: 1 *dis* = 4,5 *r*.

indirect character must be considered in order to explain all the observed phenomena.

This complexity is more pronounced in experiments with X-rays than with fast neutrons. The different spatial distribution of ionization in tissue must be responsible for this difference in the mechanism of the biological action of the two radiations. The phenomenon can advantageously be demonstrated by comparing the effect of X-rays and neutrons from equal affection of dry, dormant barley seed. In Fig. 8 such a comparison has been made by plotting the percentage and kind of affected seeds against the dose. The black areas of the two diagrams represent

the proportion of unaffected plants, *i. e.* seeds that apparently have evaded the treatment and given rise to normally fertile (> 90 % seed setting or > 80 % seed setting in the 1947 year experiment) plants not segregating chlorophyll mutations. The rest, the affected seeds, comprise non-survivors, indicated by the white areas of the diagrams, and the affected plants, represented by the grey areas. The last portion can be divided into three subgroups, *viz.* in fertile plants segregating chlorophyll mutations and partially sterile (< 90 % and 80 % seed fertility, respectively) plants with or without chlorophyll mutations in their offspring. The broken lines in Fig. 8 indicate the boundary between fertile and partially sterile plants and around these lines dotted areas show the proportions of plants giving rise to chlorophyll mutations. The horizontal extension of the diagrams is limited by the 100 per cent affection dose, and the dose scales have been chosen to give the best coincidence of the X-ray and neutron affection curves. This has been possible, as both curves seem to be exponential with an acceptable agreement, when 1 *dis* is put equal to $4{,}5$ *r*.

The most striking difference in such a comparison of the effects of X-rays and fast neutrons is the decidedly lower survival after treatments with the former radiation. This difference cannot be interpreted in pure target-theoretical terms and must be due to different degrees of indirect action, *i. e.* to different abilities in producing physiological effects. This all the more as any underestimation of the neutron relative to the X-ray induced chromosome irregularities by the simple registration of only fragments and bridges and any additional mutagenic effect, due to delayed energy dissipation after neutron irradiation, cannot influence the picture from the present basis of comparison.

The relatively higher physiological than direct point effect of X-rays when compared with that of neutrons can also be demonstrated from MARSHAK's data of 1942. He determined the frequency of abnormal anaphases in irradiated roots of *Vicia faba* at different intervals after the treatment and found the *n*/*r* efficiency not to be constant. The lowest ratio was obtained from an examination only 3 hours after treatment, which means that the observed abnormalities here must have been mainly due to physiological effects (stickiness, *etc.*). The highest values were, on the other hand, obtained from cells in the resting stage at the time of irradiation, which means that only structural changes are registered in the following anaphases. If this interpretation of MARSHAK's results be correct, the more pronounced physiological effect of X-rays is also valid for the chromosomes themselves.

When dealing with the different action of X-rays and neutrons, it is necessary to point out that a pre-soaking of the irradiated seed, which means a pronounced increase in the metabolic activity of the treated cells, does not change the obtained dissimilarity. A decidedly stronger toxic effect of the neutrons via activated water seems not to be the consequence of the hydration, as has been indicated above. This statement that fast neutrons mainly cause a genetically induced death also to metabolically active cells, while X-rays more predominantly work via physiological reactions, is interesting in connection with MITCHELL's (1947) experiments on the lethal effects produced by fast neutrons and γ-rays in mice. When giving the lethal dose with exposure times of the order of ten minutes to one hour, he found the N/γ ratio to be $7{,}5 \pm 2{,}5$, while a mean overall time of 48 hours' exposure gave a ratio of $32{,}0 \pm 8{,}6$. In addition, he found, by using the summation method, that the »mixed» radiations applied consecutively are less lethal than comparable doses of either radiation alone. As these indications on a difference in the mechanism of the biological action of fast neutrons and γ-rays were discussed from the certainly incorrect observations of SPEAR and TANSLEY (*l. c.*), any simple explanation was not to be found. If now the lethal effects of X-rays are mainly of a physiological nature, *i. e.* a certain extent of recovery is possible at long times of exposure, and the neutrons produce their lethal effects almost entirely by chromosome disturbances, *i. e.* irreversible processes, both the lower summation effect and the increased N/γ ratio with time of exposure will be easily explained. As the finding of MITCHELL that the N/γ ratio increases with time of exposure is of utmost importance in the discussion of tolerance of man to different radiations, a correct understanding of the phenomenon must have great value.

While thus an interpretation of the survival curves from the single event theory must be regarded as erroneous, there are, however, no clear objections against such a consideration on the affection curves, if only a certain parallelism be supposed to exist between the mutagenic and the toxic effect of irradiation on cells. That such a correlation really may exist has previously been shown in the present paper. The fact that the frequency/dose curves based on the proportion of surviving X_1 and N_1 plants segregating chlorophyll mutations are in agreement, if 1 *dis* has been put equal to $4{,}5\ r$ as in Fig. 8, further strengthens this conclusion. It must, however, be observed that the apparent linearity between the dose on one side and the frequency of chlorophyll mutations or affected

seeds on the other does not necessarily indicate that single hits are responsible for the observed effects (*cf.* OPATOWSKI, 1950).

The neutrons show a higher mutagenic effect than X-rays at an equal energy dissipation. Thus, several ionizations must generally be necessary to induce a mutation of the registered types. This superiority is increased, if the effect of the irradiation be first studied when a series of cell divisions has intervened. That the frequency of induced abnormalities on the whole diminishes, when the treated cells have passed through some divisions, depends on the instability or lethality of the produced irregularities but also on the direct poison or inactivation effect based on purely physiological reactions. From the present investigation we can now conclude that this increased superiority of neutrons when compared with X-rays at the later stages of plant development must be due to the high, physiologically induced lethality of the latter type of radiation. By comparing with neutrons it has thus been found that the absence of very low plant sterilities in X_1 has not been due to a genetical but to a physiological sifting. At least barley has proved to have a much higher resistance to genetic irregularities than was earlier supposed.

From a practical point of view the observed difference in the biological action of the two types of radiation should give the neutrons an apparent advantage, as the induced frequency of surviving mutations is higher here. The practical value of the produced mutations depends, however, largely on their association with high fertility. Both from the comparisons between mutated and non-mutated spikes or plants performed above, and from Fig. 8, it is clear that the obtained mutations are mainly associated with chromosomal aberrations as well in the X-ray as in the neutron experiments, at least at the doses used here. The hits have been obtained by too heavy means. In his paper of 1947 GUSTAFSSON also says that in order to produce one distinctly progressive mutation, the barley-breeder must induce 700—800 worthless types at the same time. Since the above results, as well as earlier investigations, have proved neutron-induced mutations to be even more associated with gross chromosome re-arrangements than in X-ray treatments, the obtained mutations of progressive value will certainly be of a still lower relative frequency in the neutron experiments.

There are thus strong reasons to believe that neutrons should not be superior to X-rays as a mutagenic agent when breeding diploid species. This prediction seems also to be verified by an examination of later generations. The above observation that at least the different types of chlorophyll mutations are produced in the same relative frequency

further strengthens this opinion. These remarks do not mean that neutrons with their greater possibilities to accumulate gross chromosomal re-arrangements should not be able to produce very drastic mutations to a greater extent. For polyploids with their greater tolerance to gross chromosomal interchanges no predictions can be made from the present experiments. Here research with wheat is under way.

SUMMARY.

Dry and pre-soaked seed of barley has been treated with X-rays and fast neutrons, and the biological effect of the irradiation has been followed through the first and second generation.

For dry, dormant seed the germinability was normal even after the heaviest dose applied. The retardation of the initial seedling growth was, however, pronounced, giving a sigmoid relation to X-ray dose and an exponential one to neutron dose. The frequency of mature plants, *i. e.* the survival fraction, seems to be nearly equal in X_1 and N_1 at comparable tissue doses.

The cytological response in the first mitotic cycle to irradiation showed an N/X efficiency quotient of 10, if the frequency of fragments and bridges were registered (unpublished data of Mr. N. NYBOM), while the fraction of affected seeds, the plant fertility and the rate of induced chlorophyll mutations gave N/X ratios of 65—75. These observations made at the later stage of the plant development gave to all a linear relationship to the dose. For the two last-mentioned biological events a pronounced saturation effect was, however, intervening in X_1, indicating that a contra-selection had set in. The results can be explained by postulating a physiological effect of high toxicity parallel to the genetic action of X-rays, while such a physiological killing of cells, shoots, or whole seedlings should play a subordinate rôle in neutron experiments. This interpretation is in harmony with the different spatial distribution of ionization in tissue characteristic of X-rays and neutrons, respectively.

Pre-soaking of the seed did not change this difference in the biological action of X-rays and neutrons. In X_1 the increased sensibility of the chromosomes was paralleled by an increased physiological killing. A more pronounced energy absorption due to the high moderating effect of water in the case of neutrons certainly explains why the lethality after pre-soaking in N_1 falls considerably below that of X_1.

The types of induced chlorophyll mutations were the same in X_2 and N_2, so also their relative proportions. At the high doses used in the

present study no correlation between degrees of sterility in the first generation and different categories of chlorophyll mutation in the second has been found. After both types of radiation the mutations were to a great extent associated with chromosome disturbances, and this was definitely more so with neutrons than with X-rays.

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Communications from the Swedish Seed Association. No. 49.

ABSTRACTS

SVEN O. BJÖRKMAN: Chromosome studies in *Agrostis*. (A preliminary report.)

From a cytological and taxonomical investigation in progress on the genus *Agrostis* L., some data referring to the section *Trichodium* TRIN. are given below.

A. canina L. — According to PHILIPSON (1937), two main varieties can be distinguished in the British material of this species, *viz.* var. *fasciculatis* (CURT.) SINCLAIR and var. *arida* SCHLECHT. with different ecology and shoot morphology. As to Scandinavia, all material of *A. canina* examined up to now can be divided into the same two types with few exceptions.

A. canina var. *fasciculatis* grows in damp habitats all over Scandinavia except the northernmost parts. It has intravaginal stolons. Chromosome number: $2n=14$, determined on 80 clones from Sweden, Denmark, and Finland. Plants from Great Britain, Germany, and Switzerland were also investigated. The same number is reported on Russian material of *A. canina* by SOKOLOVSKAYA (1937, 1938).

In 25 clones supernumerary chromosomes have been observed of at least two kinds, *viz.* the »normal» type (I), observed in all plants examined and having a size of about one-third of an A-chromosome (Fig. 1), and the second type (II), which has only appeared in one clone together with type I (Fig. 2) and is smaller and no doubt a derivative of the latter. In the majority of the plants examined the number of the supernumerary chromosomes is low (1 to 3), and with few exceptions this number is constant in the root-meristem cells. In P.M.C's the number normally agrees with that counted in root-tips. However, clones with a higher number of supernumeraries have shown a remarkable variation of the number in different cells. An example follows:

Clone No. 2338 (Switzerland, St. Gallen)

Number of supernumerary chromosomes	2	3	4	5	6	7	8	9	10	11	12	13
Number of cells in root No. 3 ..	1	1	3	7	6	2		1				1

The occurrence of supernumeraries — undivided or under division — between the equatorial plane and one pole at full metaphase explains this variation. In P.M.C's of the same clone, 4, 5, and 6 supernumeraries were counted (one panicle).

At meiosis the supernumerary chromosomes behave like A-chromosomes — if present in even number regularly paired and distributed; odd numbers (*e. g.* 3) can form multivalents or $s_{II}+s_{I}$, the univalents are distributed at random or divide at A I. They are also able to form multivalents with A-chromosomes.

At pollen mitosis lagging (undivided?) supernumerary chromosomes have been observed at anaphase. Whether this is to the advantage of the generative cell is difficult to say.

Studies of root-tips treated in LEWITSKY's fixative point to the possibility that the supernumerary chromosomes are fragments of an A-chromosome, but

further analysis of the idiogram and examination of the functioning of the centromere will be undertaken before the final discussion.

A. canina var. *arida* grows in rock-cracks (rocks of various composition) and in dry sandy grass-lands in Scandinavia. It has extravaginal suboles (»rhizomes»). Chromosome number: $2n=28$, counted on 99 clones from Sweden, Denmark, Finland, and Norway. Material from Iceland, Great Britain, and Germany was also examined. The same number was counted by WULFF (1937) on an *A. canina* of unknown morphology, cultivated in the Botanic Garden in Kiel. For *A. canina* in Greenland $2n=28$ is reported to me by Professor M. WESTERGAARD and Dr. TH. SÖRENSEN, Copenhagen. This material is no doubt var. *arida*.

Figs. 1—2. Root-meristem metaphases in *Agrostis canina* var. *fascicularis*, $2n=14$ + ss. — Fig. 1. No. 2338 (Switzerland, St. Gallen), nine supernumerary chromosomes of type I (one of the supernumeraries already divided). — Fig. 2. No. 960 (Sweden, Jämtland), two supernumeraries of type I and one of type II. — LEWITSKY's fixative (10 % formaldehyde + 1 % chromic acid, 1 : 1). Crystal violet. — Magnification 5200 ×.

One trisomic (pentasomic tetraploid) plant was found and three clones with supernumerary chromosomes of two kinds — one very likely identical with the »normal» type of var. *fascicularis*, the other slightly longer and of a shape very similar to supernumeraries often found in *A. stolonifera* L. and *A. tenuis* SIBTH. (sect. *Vilfa*).

The meiosis of *A. canina* var. *arida* shows a high frequency of quadrivalents — in all, 880 P.M.C's in six clones were examined, and a mean value of 4,0 quadrivalents per P.M.C. was obtained.

One plant interpreted by me as a tall *A. canina* var. *fascicularis* had the chromosome number $2n=28$, and triploid *fascicularis* specimens are also found in nature, giving support to the idea that unreduced gametes have been functioning.

Morphological transitions between the two varieties occur in nature,

though rarely. However, the chromosome counts did not show the hybrid number. Crosses in culture have been successful, and the hybrid number $2n=21$ was obtained.

The high frequency of quadrivalent formations in var. *arida*, the occurrence of polyploid *fascicularis* specimens and the transitional forms between var. *fascicularis* and var. *arida* indicate that the latter is an autotetraploid and suggest that these two varieties have a common origin.

The caryological data mentioned above will be taken into consideration in the taxonomical discussion, in which *A. canina* material from South Europe will also be included.

A. borealis HARTMAN. — Seventeen clones from Northern Sweden were investigated, all showing $2n=56$. The same number is reported by SOKOLOVSKAYA (1937, 1938) on Russian material and by BÖCHER and LARSEN (1950) on material from S. W. Greenland.

A. rupestris ALL. — Living material from three spontaneous localities in Switzerland and one locality in Poland were studied: $2n=28$; one clone investigated had one supernumerary chromosome of a size equal to the »normal» type in *A. canina*.

A. alpina SCOP. — Two spontaneous clones from Switzerland: $2n=14$.

A. Schleicheri JORDAN and VERLOT. — Two spontaneous clones from Switzerland were studied: $2n=42$.

A. setacea CURT. — Living material from two spontaneous localities in South England: $2n=14$. The same number was reported by MAUDE (1939, 1940).

A. salmantica (LAG.) KUNTH. — Seeds obtained from the Botanic Garden in Coimbra, Portugal: $2n=14$. This number was also reported by LITARDIÈRE (1950).

A. clavata TRIN. — Seven clones from three spontaneous localities in Middle Sweden (Dalecarlia, Hälsingland and Medelpad): $2n=42$. The same number was also reported by EHRENBERG (1945) on Swedish material, and by SOKOLOVSKAYA (1937, 1938) on Russian material.

A. hiemalis (WALT.) B.S.P. — Seeds obtained from one spontaneous locality in Canada (Ontario): $2n=42$. The number agrees with that reported by SOKOLOVSKAYA (1937, 1938).

A. perennans (WALT.) TUCK. — Seeds from one spontaneous locality in Canada (Ontario): $2n=42$.

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